LIFE HISTORY OF *COITOCAECUM PARVUM* CROWCROFT, 1944 (TREMATODA) FROM CANTERBURY

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ABSTRACT

The identity and life history of a trematode of the genus *Coitocaecum*, commonly found in freshwater fishes in Canterbury, has been examined. Adults collected from naturally and experimentally infected fish closely resembled *C. parvum* Crowcroft, 1944 and an account of *C. anaspidis* Hickman, 1934, given by MacFarlane (1939). They differed from the latter in the position of the genital pore, shape of the cirrus sac and ovary, and in their size. Differences between the adults from Canterbury and the types of *C. parvum* were considered insufficient to regard them as separate species.

The life history of *C. parvum* from Canterbury was completed through experimental infections of laboratory-reared hosts. Adults were recovered from the common bully, *Gobiomorphus cotidianus*, sporocysts and cercariae from the snail, *Potamopyrgus antipodarum*, and metacercariae from the mysid *Tenagomysis chiltoni*. *Coitocaecum parvum* were also found in field collections of freshwater fish (*G. cotidianus*, *G. breviceps*, *Galaxias maculatus*, *Retropinna retropinna*, *Anguilla* spp.), snails (*P. antipodarum*) and crustaceans (*T. chiltoni*, *Paracalliope fluviatilis*).

Abbreviation of the life history of *C. parvum* was found to be possible through the production of viable eggs by encysted progenetic metacercariae. Eggs of *C. parvum* metacercariae from mysids and amphipods hatched and were capable of successfully infecting laboratory-raised snails. A mechanism that may account for the release of eggs of progenetic metacercariae from within the confines of the cyst wall and tissues of the second intermediate host was examined. Metacercariae were found to excyst both on death of the mysid host and when treated with fluid from the hepato-pancreas of the host.

Some of the possible factors affecting the appearance of progenesis in *C. parvum* metacercariae have also been examined. Progenetic cysts were present in mysids during most of the year but prevalence was highest from winter to early spring and in summer. High levels in winter and spring were attributed to the gradual maturation of cysts accumulated by long-lived overwintering mysids, while high levels seen in warm summer months were probably due to the rapid maturation of cysts in spring generation mysids. The appearance of progenesis in *C. parvum* metacercariae appeared largely independent of environmental water temperature although warm temperatures

may have enhanced development. Sexual maturation and related hormonal changes in hosts were not considered important factors in stimulating egg production in *C. parvum*. The prevalence and intensity of infection with progenetic cysts did not vary between male and female hosts. Progenetic cysts were also present, although not in equal numbers, in juvenile mysids. The prevalence and intensity of progenetic infections in mysids increased with an increase in the size of the host. Although progenetic cysts were most frequently recovered from large, mature mysids, they were also occasionally found in small hosts. Physiological changes that may have occurred as the hosts aged and approached their death did not seem important in stimulating the appearance of progenesis in metacercariae. Progenesis in *C. parvum* was considered a natural result of continued growth by the metacercariae during prolonged confinement within the second intermediate host.

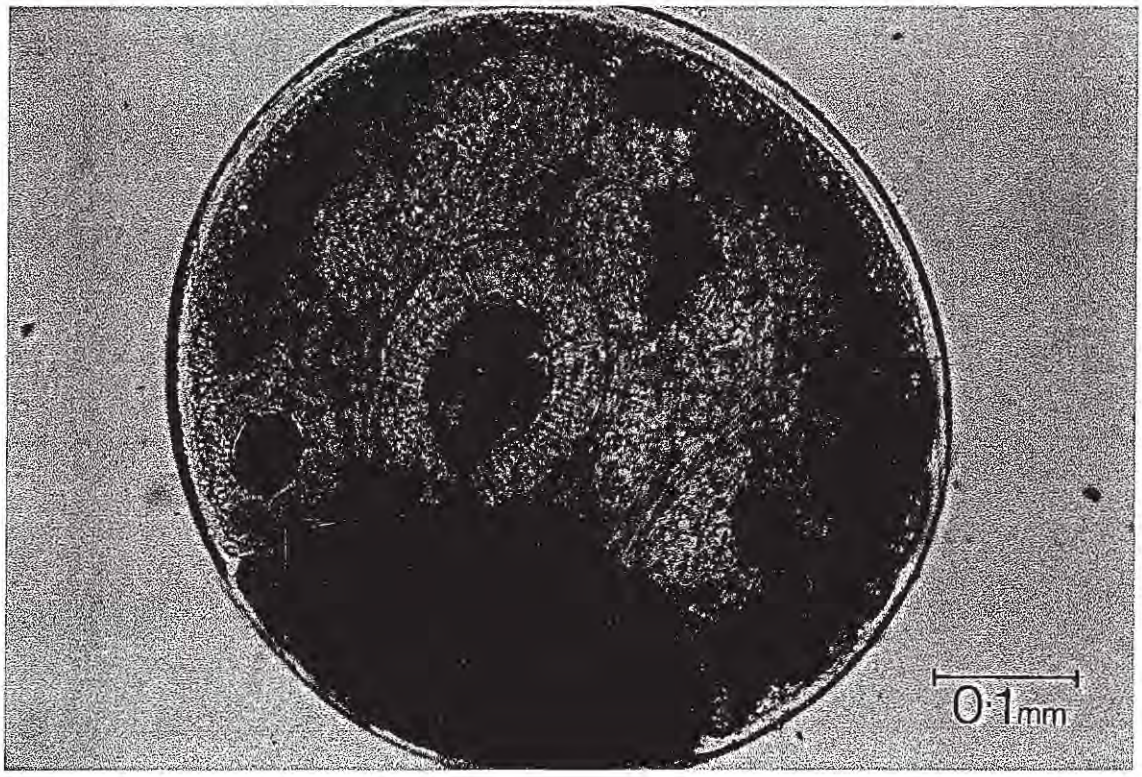


PLATE 1 Progenetic *Coitocaecum parvum* metacercaria from the mysid *Tenagomysis chiltoni* in Canterbury.

GENERAL INTRODUCTION

Very few life history studies have been carried out on the trematode parasites of freshwater fishes in New Zealand. Most of this work has been contributed by MacFarlane (1936, 1939, 1945, 1951, 1952) who described intermediate hosts for three species, *Coitocaecum anaspidis* Hickman, 1934; *Telogaster opisthorchis* MacFarlane, 1945; and *Stegodexamene anguillae* MacFarlane, 1951. It is unfortunate that this area of parasitology has been so frequently neglected as such parasites form an integral part of our freshwater ecosystems.

The species under examination in this study is *Coitocaecum parvum* Crowcroft, 1944, originally reported from New Zealand by MacFarlane (1939) and misidentified as *C. anaspidis* Hickman, 1934. Both the life history and taxonomy of *C. parvum* are discussed in the following chapters.

In order to recognise subtle differences between the species of *Coitocaecum* commonly found in Canterbury and species from elsewhere, the available literature on the history of the genus *Coitocaecum* was reviewed. By doing so, it was possible to determine which characters authors consider important in distinguishing between the various species. This information was used to aid in the identification of the New Zealand specimens.

A. HISTORY AND TAXONOMY OF THE GENUS *COITOCAECUM*

Nicoll (1915) erected the genus *Coitocaecum* for his new species, *C. gymnophallum*, recovered from the intestine of the black bream (*Sparus australis*) in Australia. Characteristics of the genus included the fusion of the intestinal caeca (a feature, at this time, unique to *Coitocaecum*), the apparent lack of a *true* cirrus sac and the absence of a seminal receptacle. Nicoll, unsure of the family status of the genus, believed it to most resemble the Allocreadiidae. However, he considered these unique features of *C. gymnophallum* were enough to exclude it from this family. He made no further attempt to assign the genus to a family.

During the following years several more species of *Coitocaecum* were described. Three major trends appeared in the classification of the genus depending which characters authors considered significant in digenean taxonomy.

The current trend is to follow Ozaki (1925) and place *Coitocaecum* in the family Opecoelidae (Crowcroft, 1951; Yamaguti, 1971; Hine, 1977; Ahmad, 1980). Ozaki (1925) erected the genus *Opecoelus* for two new species. Members of this genus closely resembled *Coitocaecum*, differing only in the presence of an anus and marginal extensions of the acetabulum in *Opecoelus*. Ozaki considered the absence of a seminal receptacle and fusion of the caeca, found in both genera, significant and excluded them from the Allocreadiidae. In 1925 Ozaki erected a new family for the genera, the Opecoelidae; however, Ozaki (1926, 1929) reconsidered his earlier move, having described a further five new species of *Coitocaecum*. He now considered that the absence of an anal canal and anus in *Coitocaecum* warranted the erection of a new family, the Coitocaecidae, for the genus. The move to put *Coitocaecum* in a family of its own as proposed by Ozaki (1929) does not seem well founded and has only been followed by Wisniewski (1933) and Hickman (1934).

The second trend amongst authors has been to include *Coitocaecum* in the Allocreadiidae, sub-family Opecoelinae (Stunkard, 1931; Manter, 1934; Harshey, 1937; Crowcroft, 1944). Stunkard (1931) considered the removal of *Coitocaecum* from the Opecoelidae by Ozaki (1929) as unwarranted. In his opinion, the presence or absence of anal openings in the trematodes was not a significant taxonomic character. Instead, Stunkard favoured the retention of *Coitocaecum* with *Opecoelus* and *Anisporus* in the Opecoelidae but thought it more useful to reduce this family to sub-family status and include it with the Allocreadiidae.

The third classification scheme has been to follow Poche (1925) and place *Coitocaecum* in the family Allocreadiidae, sub-family Coitocaecinae (Pigulevsky, 1932; Woolcock, 1935; MacFarlane, 1939; Park, 1939; Skrjabin, 1958). Poche (1925) considered that the fused caeca, lack of cirrus sac, and absence of the seminal receptacle did not justify exclusion of *Coitocaecum* from Allocreadiidae. Since the genus did not resemble any of the known sub-families, Poche erected the Coitocaecinae for it. In 1935 Woolcock added her new genus, *Dactylostomum*, to the Coitocaecinae. Both *Coitocaecum* and *Dactylostomum* were similar in morphology but the latter was distinguished from *Coitocaecum* by protuberances on the acetabulum.

In 1933, Wisniewski subdivided the genus *Coitocaecum* into three genera, *Nicolla*, *Ozakia*, and *Coitocaecum*. Members of *Nicolla* were

characterised by the central position of the genital pore and the presence of a seminal receptacle. In addition, the seminal vesicle was entirely closed within the cirrus sac. In *Ozakia* the genital pore was positioned to the left of the body, the seminal receptacle was absent, and only the terminal portion of the seminal vesicle was enclosed within the cirrus sac. *Ozakia plagiorchis* (=*C. plagiorchis* Ozaki, 1926) became the type species for this genus. The genus *Coitocaecum* was left with its type species, *C. gymnophallum*, *C. proavitum* Wisniewski, 1933, and *C. testiobliquum* Wisniewski, 1932. In this genus the genital pore opened to the left of the body and a seminal receptacle was absent as in *Ozakia*. The terminal portion of the seminal vesicle was enclosed by a "retort-shaped" cirrus sac (Crowcroft, 1951; Yamaguti, 1971).

In 1947 Manter transferred several other species of *Coitocaecum* to *Ozakia*, including *C. anaspidis*.

Crowcroft (1951) considered the splitting of *Coitocaecum* by Wisniewski as premature as there was insufficient evidence of any real differences existing between the genera. This was especially so with *Nicolla* which contained only two species. On re-examination of *C. gymnophallum* prepared by Nicoll (1915), Crowcroft noted that his sections "revealed the presence of a small membranous cirrus sac enclosing a short terminal portion of the male duct". He presumed that Nicoll had observed this small cirrus sac but, in light of contemporary definition of this structure, Nicoll had not considered it a *true* cirrus pouch [Crowcroft's italics]. Crowcroft concluded that the condition of the cirrus sac in *C. gymnophallum* was consistent with the diagnosis of *Ozakia* given by Wisniewski (1934). As *C. gymnophallum* was the type species for *Coitocaecum*, Crowcroft recommended that *Ozakia* fall, and all species placed in it be transferred back to *Coitocaecum*.

Both Slusarski (1958) and Yamaguti (1958) considered *Coitocaecum*, *Nicolla* and *Ozakia* synonyms. Yet, in spite of this, Yamaguti (1970) transferred four more species to *Ozakia* and in 1971 renamed *Coitocaecum parvum* as *Ozakia parvum*.

Coitocaecum is very similar to members of the family Allocreadiidae. Nicoll (1915) believed that the genus could not be included in this family because of the lack of a cirrus sac in *C. gymnophallum* and the fusion of the intestinal caeca. Crowcroft (1951) observed a small cirrus sac in his

sections of *C. gymnophallum*. It should be pointed out that there are now many genera of trematodes known which contain species with fused caeca (e.g., *Cyclocoelum* Brandes, 1892; *Cycloprimum* Witenberg, 1923; *Haematoprimum* Stossich, 1902). This condition is no longer restricted to *Coitocaecum* and therefore cannot be considered a significant taxonomic character. For these reasons, *Coitocaecum* has been assigned, in this study, to the family Allocreadiidae, sub-family Coitocaecinae. Even though *Coitocaecum* and *Opecoelus* bear close resemblance to one another, the absence of an anal canal and anus is probably enough to exclude *Coitocaecum* from the sub-family Opecoelinae.

It is clear from this review that there is no general agreement amongst authors as to the placing of the genus *Coitocaecum* within the Digenea. In addition to this problem there appears to be no single consensus over which specific characters should be used in identifying between species of *Coitocaecum*. The characters used in this study are outlined on page 38 (Chapter I).

B. LIFE HISTORY AND DEFINITIONS OF PROGENESIS

Although there are at least 28 members in the genus *Coitocaecum*, complete or partial life histories are only known for three, *C. plagiorchis* Ozaki, 1926, *C. anaspidis* Hickman, 1934, and *C. parvum* (=*C. anaspidis* Hickman, 1934, of MacFarlane, 1939). Partial life histories are known for a further three closely related species, *Nicolla skrjabini* (Ivanitskii, 1928) Wisniewski, 1934; *N. gallica* (Dollfus, 1941) Dollfus, 1959; and *N. testiobliquum* (Wisniewski, 1932) Wisniewski, 1934. Typically, three hosts are utilised in the life histories of these species. Adults are found in the intestine or gall bladder of freshwater fishes, sporocysts in molluscs, and metacercariae in crustaceans. It is perhaps interesting to note that in four of these species, and in *C. parvum*, the species examined in the present study, some metacercariae become gravid while encysted within the crustacean host. This phenomenon is termed progenesis and, although not rare in other trematodes, is considered an evolutionary oddity by some authors (McMullen, 1938; Grabda-Kazubska, 1976).

Progenesis is a term frequently used to describe the precocious sexual maturity of metacercariae in species such as *C. parvum* examined in this study. Unfortunately, there is no single consistent definition

for progenesis in the literature which has undoubtedly led to much confusion over usage of the term. In order to avoid ambiguity, some of these definitions are discussed in the following section.

Progénèse was initially coined by Giard (1887), the precise meaning of this term is unclear, and there are now at least two interpretations of his original definition. Stunkard (1970) took it to mean "sexual maturity of animals that had not yet attained the adult condition", while Jamieson (1966a) interpreted it as "precocious maturity in animals where this was associated with arrested development". In addition to this problem, many authors consider progenesis to be synonymous with neoteny (De Beer, 1958; Jamieson, 1966b; Pearson, 1972; Smyth, 1976). For the purpose of discussions in this thesis, definitions given by Gould (1977) and Mackiewicz (1981) are followed. Gould (1977) defines progenesis as, "paedomorphosis, produced by *precocious* sexual maturation of an organism still in a morphologically juvenile state". Neoteny, on the other hand, is "paedomorphosis, produced by *retardation* of somatic development" (Gould, 1977, page 485).

The essential difference between the two is the process by which they come about, progenesis by early sexual maturity and neoteny by retardation of somatic development, although the end products may appear similar (Mackiewicz, 1981). Of these two definitions, the situation in *C. parvum* most closely resembles progenesis. In progenetic *C. parvum* metacercariae there appears to be no retardation of somatic development. The reproductive, digestive and excretory systems are not completely formed in newly encysted metacercariae but are all well developed by the time metacercariae reach egg production.

Another point, which perhaps needs to be defined, frequently arises in discussions of progenesis. Many authors consider that metacercariae should be referred to as *adults* once egg production has begun (Buttner, 1955; Jamieson, 1966b; Pearson, 1972), while others prefer to call them *progenetic metacercariae* (Hickman, 1934; MacFarlane, 1939; Grabda-Kazubska, 1976; Font, 1980; Mackiewicz, 1981). Mackiewicz (1981) suggested that a definition of an adult or juvenile parasite may not necessarily be based purely on morphology but may also refer to the part of a life history in which the parasitic stage occurs and the habitat it occupies. Freeman (1973) pointed out that a characteristic of an adult cestode is the enteral habit, whereas the metacestode (juvenile) is parenteral. Further, a

parasite may be considered a functional adult in an invertebrate host only when worms are unencysted (a cyst wall is considered a juvenile character of trematodes) and there is an efficient means for egg dispersion. In some species, eggs from progenetic metacercariae remain encased within the cyst wall of the trematode and can only be released on death of the intermediate host. In this case, these metacercariae may be considered pre-adults or progenetic metacercariae as there is no efficient means of egg dispersal. Some trematodes are found in invertebrate hosts, unencysted and inhabiting sites where their eggs are easily dispersed, e.g., in the antennary gland of crayfish (Font, 1980) or mantle cavity of cephalopod molluscs (Short and Powell, 1968). These trematodes are considered fully functional adults.

C. AIMS OF THE PRESENT STUDY

The aims of this thesis are threefold. The first is to establish the identity and life history of *Coitocaecum parvum*, a trematode commonly found in freshwater fishes in Canterbury. The second is to determine whether or not an abbreviated life history is possible for this species through progenesis of the metacercariae, and the third to examine if water temperature, host sex, host length, or age of metacercariae are important factors in the manifestation of progenesis in *C. parvum*.

Although two species of *Coitocaecum* are found to occur in Canterbury, only *C. parvum* is studied in this thesis. The reasons for this are as follows: (1) all hosts of *C. parvum* are available in large numbers throughout the year; (2) metacercariae of *C. parvum* are known to be progenetic which led to some interesting questions concerning a possible abbreviated life history in this trematode; and (3) the second species, *C. zealandicum* (Hine, 1977), occurs only in torrent fish which are not easily obtained by conventional methods of capture. In addition, no other life history stages are known for *C. zealandicum*.

CHAPTER I

THE LIFE HISTORY AND TAXONOMY OF *COITOCAECUM PARVUM* CROWCROFT, 1944, FROM CANTERBURY

1.1 INTRODUCTION

Two species of *Coitocaecum* have been described from New Zealand. The first, identified as *C. anaspidis* Hickman, 1934, by MacFarlane (1939), was found in freshwater fish in Canterbury. *Coitocaecum anaspidis* had been originally described as progenetic metacercariae from crustacean hosts in Tasmania (Hickman, 1934). The second species, *C. zealandicum* Hine, 1977, was described from the torrent fish (*Cheimarrichthys forsteri* Haast, 1874) collected from the Wellington region.

During the present study, preliminary work involving the examination of seven freshwater fish species in Canterbury revealed two distinct species of *Coitocaecum*. One of these, found only in the intestine of the torrent fish, was clearly *C. zealandicum*, however the identity of the other species, collected from bullies, Inanga and smelt, was not immediately obvious. This second form appeared to represent the species MacFarlane (1939) identified as *C. anaspidis* Hickman, 1934. However, the genus *Coitocaecum* has expanded considerably since MacFarlane's work. A review of its members and examination of the types of *C. anaspidis* and *C. parvum* indicated that both MacFarlane's form and the specimens found in this study were in fact not *C. anaspidis*. Rather, they should be referred to as *C. parvum* Crowcroft, 1944, described from a galaxiid fish (*Galaxias attenuatus* Jenyns, 1842) in Tasmania. The taxonomy of the forms involved is discussed later.

MacFarlane (1939) gave brief descriptions of the forms he presumed to be the larval stages of his *C. anaspidis* (=*C. parvum*). The molluscan hosts were *Potamopyrgus antipodarum* Gray, 1843, and *P. badia* (=*P. antipodarum* Gray, see Winterbourn, 1970), and the second intermediate host the amphipod *Paracalliope fluviatilis* Thomson. A small proportion of metacercariae in the amphipod host exhibited progenesis.

The proposal of a life history based purely on anatomical similarities between the larval stages can be considered dubious. This is

especially true when two closely related species, such as *C. zealandicum* and *C. parvum*, occur in the same region. Larval stages of members of a single genus are also likely to be closely related in form and morphological detail (Yamaguti, 1971), for example, at least two other species of *Coitocaecum*, not found in New Zealand, have microcotylocercous cercariae and progenetic metacercariae (Wisniewski, 1933; Dollfus, 1938, 1959). In addition, the life history of a single species of Digenean is both complex and divergent (Stunkard, 1957). Miracidia and cercariae often bear little resemblance to the final adult stage (Mayr, 1969). Reproductive and digestive systems are not usually differentiated. Although larval characters such as tails, stylets and penetration glands may be present, these quickly disappear on penetration of the next host (Erasmus, 1972; Whitfield, 1979; Chappell, 1980). The relationship between larval stages and adult flukes can be established only by experiment (Mayr, 1969).

The problems of identification of the larval stages were overcome, in this study, by rearing *C. parvum* through laboratory raised, experimentally infected hosts. The life history stages in naturally infected hosts were identified by morphological comparison with the stages obtained through experiment.

To summarise, the aims of this chapter are to study the life history of *C. parvum* under both natural and experimental conditions and to discuss its identity by comparison with other members of the genus.

1.2 METHODS

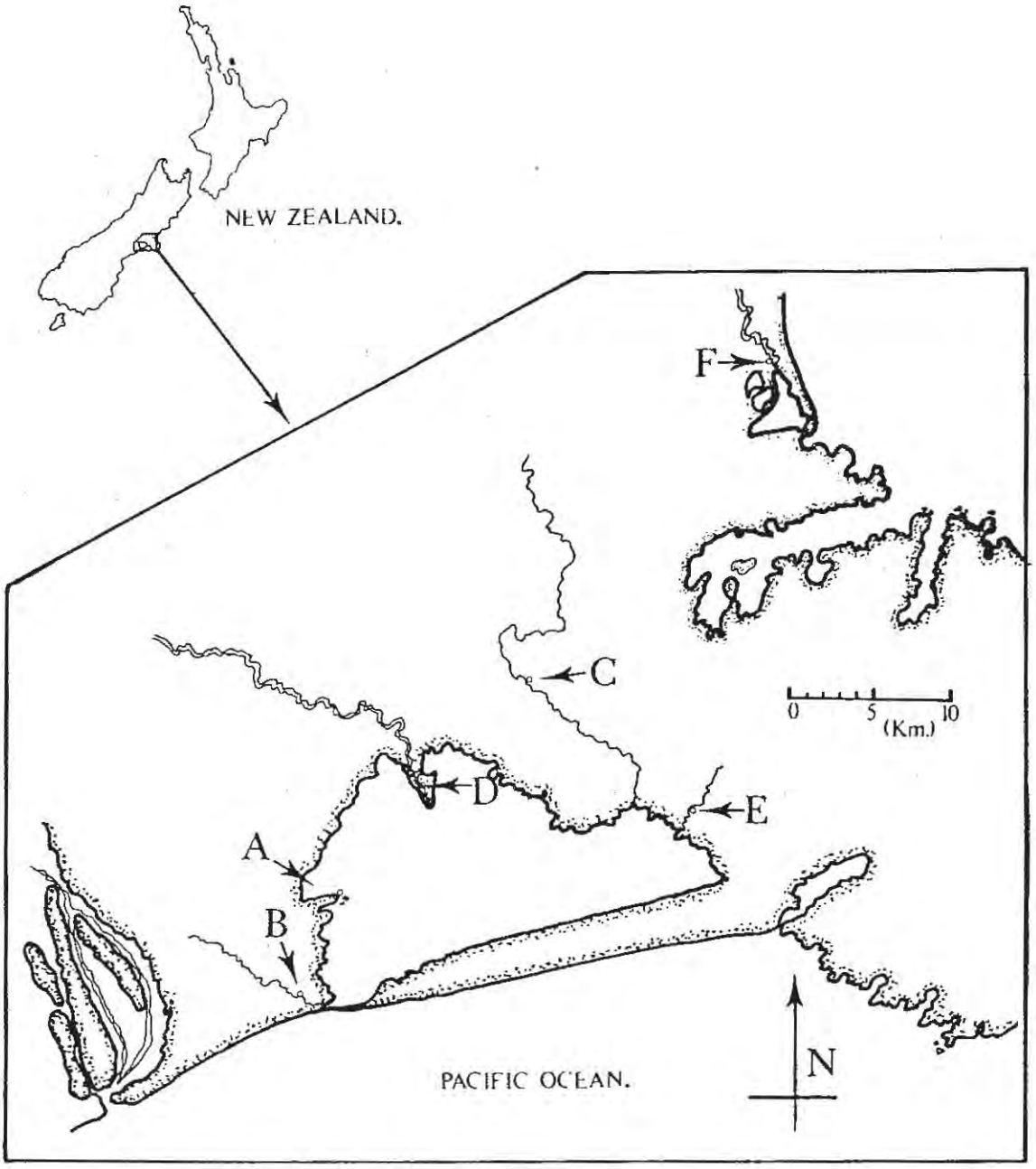
1.2.1 Study Site

Hosts were collected from several areas around the Canterbury region. Definitive fish hosts, crustaceans and gastropods were obtained from Timber Yard Point, Lake Ellesmere; the lower reaches of the Selwyn River; Taumatu Stream; Halswell River; Avon-Heathcote Estuary, and the Ashley River. These sites represented a range of habitats for potential hosts and for parasites. Major study sites are given in Fig. 1.1.

1.2.2 Completion of the Life History

The life history of *C. parvum* was determined experimentally by infecting laboratory-reared hosts with parasite larval stages of known

Fig. 1.1 A map of Lake Ellesmere and surrounding regions indicating major sampling sites. A - Timber Yard Point; B - Taumatu Stream; C - Halswell River; D - Lower Selwyn River; E - Queens Stream; F - Lower Avon River.


NEW ZEALAND.
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ancestry. The stages obtained from experimental infections were compared with those occurring in naturally infected hosts.

(a) Collection and maintenance of hosts

(i) Fish. Three species of fish, the common bully (*Gobiomorphus cotidianus* McDowall, 1975), the brown trout (*Salmo trutta* Linnaeus, 1758), and the quinnat salmon (*Oncorhynchus tshawytscha* Walbaum, 1792), were collected for use in infection experiments.

Forty recently hatched common bullies were collected from the Halswell Stream during February 1982. Twenty bullies were dissected on return to the laboratory and examined for parasites. No parasites were found. The remaining 20 fish were placed in an aquarium and fed tubifex worms. After 10 months they were used in infection experiments.

One hundred and thirty brown trout fry were collected from the Glenariffe Stream trap (supplied by the Ministry of Agriculture and Fisheries, Freshwater Division). On return to the laboratory, 65 of these were killed and examined for parasites. No *Coitocaecum* were found. The remaining fish were placed in an aquarium at 15°C and fed tubifex worms. After four months, only 20 fish were still alive. These parasite-free fish were used in infection experiments.

Thirty young salmon fry were collected from the Lake Coleridge South Pacific Salmon Hatchery. On return to the laboratory, 15 of these fish were dissected and examined for *Coitocaecum*. No parasites were found. The remaining fish were maintained in an aquarium to be used in later infection experiments.

(ii) Snails. Thirty large snails of the species *Potamopyrgus antipodarum* were collected from the Halswell Stream in June 1981 and were maintained in the laboratory for 18 months. They were kept in a bucket with distilled water, washed *Elodea* and chalk (following recommendations by Pearson, 1972; Kuris, 1980a). During this time the snails released young which fed and slowly matured. Once the young snails had reached a suitable size (over 1 mm) they were ready for use in the infection experiments.

(iii) Crustaceans. Thirty mature amphipods of the species *Paracalliope fluviatilis* were collected from Taumatu Stream to provide the basis of a laboratory-reared population. Amphipods were maintained in an aquarium for eight months (temperature 15-20°C). Fish pellets were administered at regular intervals although it is not sure if amphipods were feeding on these. Young released by adult amphipods were maintained in the aquarium until they had reached a size of 2 mm (MacFarlane [1939] noted that amphipods under this size were not infected with *C. parvum* in nature).

Attempts were made to raise mysids (*Tenagomysis chiltoni* Tattersall, 1923) in the laboratory by isolating adult females with embryos in the marsupium in aquaria, however, rearing of young mysids was not successful.

(b) Sources of *Coitocaecum parvum* and infection of hosts

A series of four infection experiments was designed to complete the life history of *C. parvum*, a summary of pathways followed is presented in Fig. 1.2. Sources of *C. parvum* used in the infection of hosts are given with each experimental procedure.

(i) Experiment 1. In this experiment, cercariae of *C. parvum* used to begin the life history of *C. parvum* in the laboratory were collected from naturally infected snails. All cercariae used to infect amphipods were collected fresh shortly after their emergence from the snail host.

Groups of five cercariae were added to watch glasses each containing one laboratory-bred amphipod. After 24 hours the amphipods were transferred to a Petri dish with water and food. A total of 48 amphipods was infected in this manner. A control group consisting of 20 amphipods was collected from the same source as the test group (laboratory-bred in an aquarium), dissected, and examined for parasites. None was found.

The group of 48 laboratory-infected amphipods was then fed to three laboratory-raised common bullies. Each fish was isolated overnight in a small vessel containing 16 amphipods. The following morning the vessel was checked to confirm that all amphipods had been eaten and the fish were returned to an aquarium. After four weeks the fish were killed by decapitation and the intestines examined for the presence of parasites.

The control for this experiment consisted of six laboratory-raised bullies which were killed and examined for parasites. None was found. Ovigerous adults were collected from infected fish and maintained in saline (0.75% NaCl in distilled water) for 24 hours. Seven eggs, which passed out of the uterus of these worms, were collected from the bottom of the watch glass and transferred to distilled water where they continued to develop. After four days the eggs were placed in a dish with ten laboratory-bred snails. After a further seven days the snails were transferred to a Petri dish containing distilled water and washed *Elodea* stems. Chalk was also supplied as a source of calcium for continued shell growth (Kuris, 1980a). Two weeks later the snails were crushed and their tissues examined for sporocysts of *C. parvum*.

(ii) Experiment 2. This experiment differed from Experiment 1 in two ways. Firstly, the intermediate host used to infect fish was the mysid *T. chiltoni*. Secondly, mysids were not laboratory-bred but collected from the field. *Coitocaecum parvum* metacercariae were derived from natural infections of this host.

Twenty brown trout were used in this experiment. Five of these trout were dissected prior to experimentation and examined for the presence of parasites. None was found. The remaining 15 fish were fed infected, field collected mysids *ad lib.* for one week. After this time they were returned to a normal diet of tubifex worms for a further two weeks. At the end of this period the fish were removed from the tank and killed. The intestines were removed and examined for parasites.

The procedure outlined above was repeated using salmon fry. Fifteen fish were used in this experiment. Five were dissected as controls prior to experimentation. The remaining ten were fed naturally infected mysids *ad lib.* and killed after two weeks.

As trout and salmon fry did not become infected with *C. parvum*, eggs used to infect snails were obtained from laboratory-raised bullies as follows. Eight laboratory-raised bullies were fed naturally infected mysids *ad lib.* for one week. After this time bullies were returned to their normal diet of tubifex worms for a following two weeks. At the end of this period bullies were killed by decapitation, the intestines removed, and parasites collected. The control fish used in this experiment were

also used as controls in Experiment 1; none contained parasites.

Nineteen ovigerous adult *C. parvum* collected from these fish were kept in 0.75% NaCl in distilled water for 24 hours. Forty-seven eggs were collected from the bottom of the watch glass the following morning. These were transferred to a dish of distilled water where they continued to develop for four days. After this time had elapsed, ten laboratory-raised snails were also placed in the dish, along with washed *Elodea* and a small piece of chalk. Two weeks later the snails were crushed and their tissues examined for sporocysts of *C. parvum*.

(iii) Experiment 3. Attempts to raise mysids in the laboratory proved unsuccessful. For this reason unparasitised specimens were collected by visual examination of live mysids. Two hundred mysids (*T. chiltoni*) were collected from Queens Stream, Kaituna Valley (Fig. 1.1) during April 1982. Only 26 out of 100 of these mysids examined contained cysts of *C. parvum*. The remaining 100 mysids were each placed in a watch glass with water and examined under a dissecting microscope (x 16 magnification) Metacercarial cysts in infected individuals were easily seen through the transparent carapace of the mysid. Mysids which were observed with cysts were discarded. The remaining 78 mysids were used in this experiment. A control group composed of 44 of these mysids was immediately dissected and further examined for cysts. None were found. The remaining 30 mysids were placed in a container with 15 naturally infected snails known to be shedding *C. parvum* cercariae. After one week the mysids were killed and the number of metacercarial cysts present recorded.

(iv) Experiment 4. This experiment involved the infection of laboratory-bred snails with miracidia from adult *C. parvum* from naturally infected fish hosts.

To obtain eggs, bullies (*Gobiomorphus cotidianus*) were collected from the Taumatu Stream (April 1982). On return to the laboratory live fish were placed in a small bucket with a little water and kept overnight. The next morning faeces were collected from the bucket and removed to a jar containing distilled water. The faeces were broken up by vigorous shaking of the jar. The resulting slurry was poured through a nest of sieves (Endecott's Test Sieves, London), and washed with distilled water.

Eggs of *C. parvum* were retained in the 37 µm sieve. The sieve was washed out and its contents sedimented following the method of Orr and Hopkins (1969). Immediately after faeces had been collected the fish were killed and their intestines examined for parasites. As only *C. parvum* was recovered, it was concluded that the eggs collected from the faeces came from this parasite.

Thirty laboratory-bred snails were isolated in a Petri dish containing 74 eggs which had been collected from the faeces of the naturally infected fish. Snails remained in the Petri dish for four months, with a supply of washed *Elodea* leaves. Snails were frequently observed grazing over the surface of the leaves. It is presumed they were feeding on algae growing on the leaves. Every two weeks the Petri dish was examined under a dissecting microscope for the presence of cercariae. After four months the experiment was terminated and the number of infected snails determined. Snails that were not shedding cercariae were crushed and their tissues examined for the presence of an infection. The control group for this experiment was composed of 20 snails which had been taken from the breeding bucket and killed. No parasites were found in these snails.

A summary of these infection experiments is presented in Figure 1.2.

1.2.3 Potential Field Hosts

(a) Fish

Seven species of fish were caught and examined for the presence of *C. parvum* and *C. zealandicum* during 1981-1982. These are listed in Table 1.1, along with the number of fish collected, site, and method of capture. Identification of the species caught was based on McDowell (1978).

(b) Snails

At least five species of freshwater gastropod inhabit the lakes and streams where infected crustacean and fish hosts occur. Samples of these were collected and examined for the presence of larval stages of *C. parvum*. A species of brackish water gastropod was also collected (Table 1.2). Identification of the snails was based on Winterbourn (1970a, 1980).

Fig. 1.2 A summary of infection experiments designed to complete the life history of *Coitocaecum parvum* in the laboratory. Naturally infected hosts (left), experimentally infected (right). Experiment 1 (solid line), Experiment 2 (arrows), Experiment 3 (dotted line), Experiment 4 (broken line).

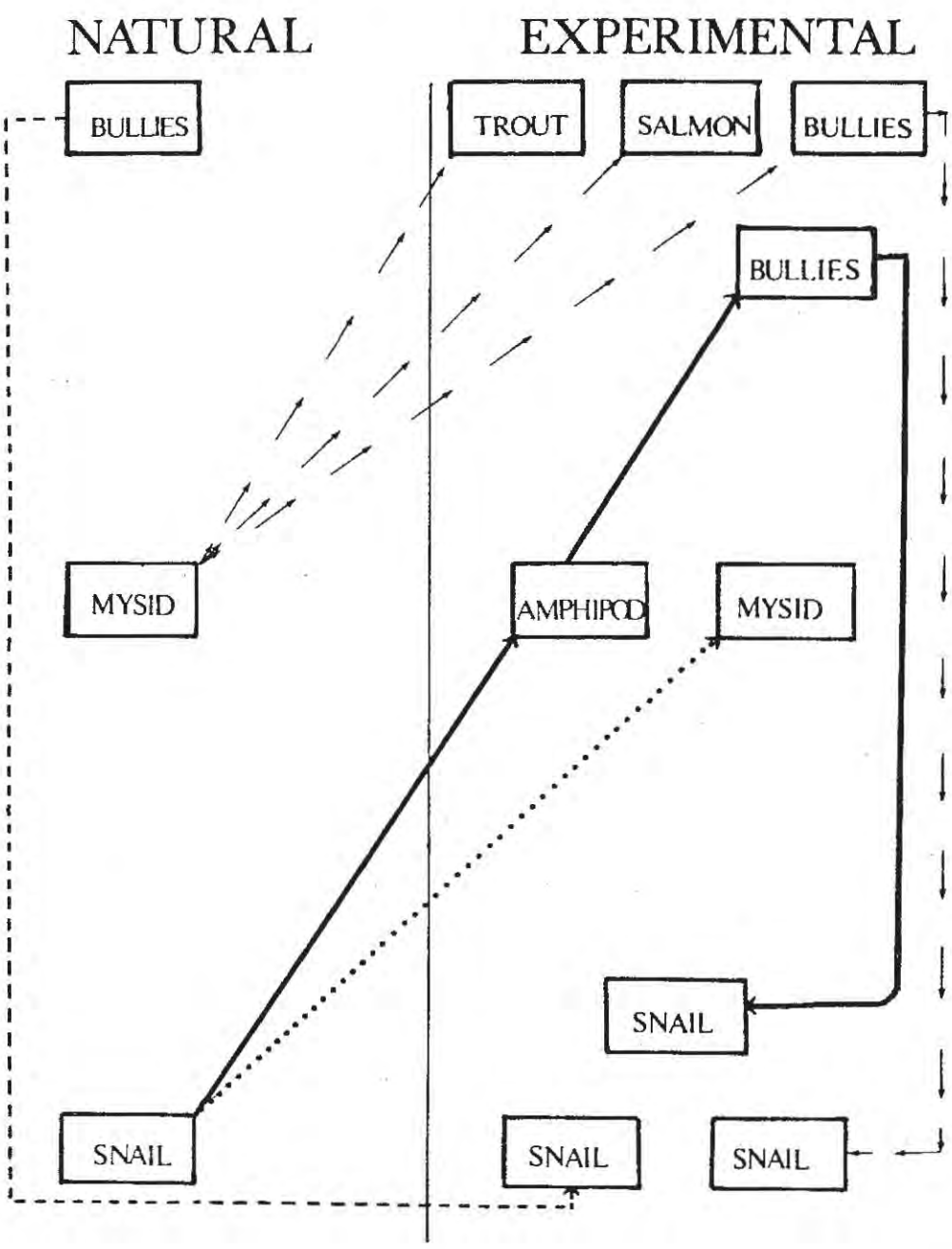

NATURAL
EXPERIMENTAL
BULLIES
TROUT
SALMON
BULLIES
BULLIES
MYSID
AMPHIPOD
MYSID
SNAIL
SNAIL
SNAIL
SNAIL

Table 1.1 Fish species sampled as potential hosts for *Coitocaecum parvum* and *C. zealandicum*.

Species	Common Name	N=	Method of Capture	Site
Gobiomorphus cotidianus	Common bully	90	Hand-net, seine	Timber Yard Point, Taumatu and Halswell Stream
Gobiomorphus breviceps	Upland bully	15	Hand-net	Lake Clearwater
Galaxias maculatus	Inanga	12	Seine	Taumatu Stream
Retropinna retropinna	Common smelt	57	Hand-net, seine	Timber Yard Point, Taumatu Stream
Carassius auratus	Goldfish	18	Hand-net	Halswell Stream
Anguilla spp.	Eels	38	Fyke net	Timber Yard Point
Cheimarrichthys forsteri	Torrent fish	3	Electric fishing apparatus	Ashley River

Table 1.2 Molluscs sampled as potential first intermediate hosts for *Coitocaecum parvum*.

Species	Habitat	N=	Method of Capture	Site
Potamopyrgus antipodarum	Fresh water	2758	Hand-net	Timber Yard Point, Taumatu Stream, Halswell River
Potamopyrgus estuarinus	Brackish water	113	Towed net	Avon-Heathcote Estuary, Lower reaches of Avon River
Lymnaea tomentosa	Fresh water	17	Hand collected	Shipleys Creek, Taumatu Stream
Gyraulus corinna	Fresh water	57	Hand-net	Taumatu Stream
Physastra variabilis	Fresh water	38	Hand-net	Taumatu Stream
Physa sp.	Fresh water	157	Hand-net	Taumatu Stream

(c) Crustaceans

Six species of crustaceans from the Canterbury area were collected and examined for metacercariae of *C. parvum*. These are presented in Table 1.3, along with the site and method of capture. Identification of the crustaceans was based on Chapman and Lewis (1976).

In addition, a small sampling programme was conducted on the mysid *Tenagomysis chiltoni* and the amphipod *Paracalliope fluviatilis*. Comparisons were made of the differences in the prevalence and intensity of infection with progenetic metacercariae of *C. parvum* from these two crustaceans. Between March and July 1982, five samples of mysids and amphipods were collected from the Selwyn River. The lower reaches of the Selwyn River was the only area known where both mysids and amphipods could be collected in any numbers from the same habitat. Mysids and amphipods were caught in a hand-net (mesh size 0.25 mm) drawn across the weeds 0.5 metres from the shore. A mesh size of this diameter ensured that the smallest mysids and amphipods were collected in the sample. On return to the laboratory the crustaceans were killed by decapitation and examined for cysts of *C. parvum*. The site, prevalence, and intensity of infection was recorded. Metacercariae were classified as either egg-bearing or immature. The number of eggs present in each cyst was counted and the state of development of the eggs was recorded.

Statistical tests used to determine significant differences between the results were Student's t-test, chi-squared analyses, and Wilcoxon's two sample test for non-normal distributions (Muzzall, 1980). Differences were considered different at $P < 0.05$.

To avoid confusion, prevalence and intensity of infection are defined as follows. Prevalence is the percent of infected hosts in a given sample. Intensity is the mean number of worms per infected host (Muzzall, 1980; Margolis *et al.*, 1982).

1.2.4 Treatment of Worms

(a) Collection and treatment of worms

(i) Adults. Fish brought in from field collections were examined for parasites one or two days after return to the laboratory. Both experimentally and naturally infected fish were killed by decapitation.

Table 1.3 Crustaceans sampled as potential second intermediate hosts for *Coitocaecum parvum*.

Species	Common Name	N=	Method of Capture	Site
Tenagomysis chiltoni	Mysid	227	Hand-net	Timber Yard Point; Queens Creek, Kaituna Valley; Selwyn River
Tenagomysis macropsis	Mysid	79	Boat-towed net	Lower reaches of Avon River
Paracalliope fluviatilis	Amphipod	143	Hand-net	Lake Ellesmere, Selwyn River, Halswell Stream, Taumatu Stream
Paracorophium luscasi	Amphipod	52	Hand-net	Timber Yard Point, Lake Ellesmere
Paratya curvirostris	Freshwater shrimp	35	Hand-net	Avon River
Paranephrops zealandicum	Freshwater crayfish	4	Hand-net	Small stream feeding Lake Ellesmere

The intestine and associated organs were removed to a Petri dish containing saline. The intestine was slit lengthwise and any parasites present removed from the intestine wall.

Adult flukes were either examined live or fixed in hot 10% formalin and stored in 70% alcohol. Some specimens of *C. parvum* were stained in Delafield's Haemotoxylin or Gower's carmine and mounted in Canada Balsam. Measurements given in the text, unless otherwise stated, are from ovigerous specimens seen in ventral view. The mean, in millimetres, is followed by the standard deviation and number of observations upon which measurements were based.

Serial sections (6-8 µm) were cut from prepared specimens of *C. parvum*, stained in Delafield's Haemotoxylin, counter-stained in Eosin and mounted in Eukitt.

(ii) Sporocysts and cercariae. Snails were killed by crushing the shells between a pair of fine forceps. Care was taken not to damage the delicate parasites inside. The internal viscera were teased out into saline and examined for the sporocysts of *C. parvum*. Sporocysts were either studied live with vital stains or fixed in hot 10% formalin.

To collect cercariae, experimentally infected snails were placed in a watch glass with a small amount of fresh water. Freshly emerged cercariae were collected from the bottom of the watch glass after about 30 minutes. (Room Temp. 15-20°) Cercariae used in morphological work were studied live, unstained or stained in dilute neutral red. Ten cercariae were measured live in water under slight coverslip pressure. Ten cercariae were fixed in hot 10% formalin and measured. Measurements given in the text are from these fixed specimens.

(iii) Metacercariae. Crustaceans were killed by decapitation by severing the head from the thorax with a pair of fine scissors. The internal organs were teased out into saline and examined for encysted metacercariae. The remaining parts of the crustaceans, such as legs and abdomen, were also examined.

Before fixation, metacercariae were excysted by gently rupturing the cyst wall with a blunt-ended needle. On release from the cysts

metacercariae were observed to undergo vigorous movements indicating that no damage had been inflicted upon them by this method of excystation.

Metacercariae were either fixed in hot 10% formalin or examined live. Some fixed specimens were stained in Delafield's Haemotoxylin or Gower's carmine and mounted in Eukitt.

Two sets of measurements are given in Table 1.8. The first represents measurements taken from ovigerous metacercariae and the second from immature specimens.

(b) Museum specimens

The identity of *C. parvum* was determined by comparing laboratory and field collected adults with original descriptions of *Coitocaecum* spp. and with prepared slides. For this work, the following specimens were borrowed from various museums and institutions.

Coitocaecum anaspidis Hickman, 1934.	Helminth Coll., T.M. Two syntype microslides prepared by Professor V.V. Hickman. Reg. No. K548 bearing two whole mounts from *Anaspides tasmaniae*. K550 bearing two whole mounts. Slides with serial sections.
Coitocaecum anaspidis Hickman, 1934.	National Parasite Collection, U.S.D.A. Collected by H. Manter, 1954 from *Anguilla dieffenbachii* in N.Z. Species No. 49164 in U.S.N.M.H.C.
Coitocaecum parvum Crowcroft, 1944.	Helminth. Coll., A.M. One co-type slide prepared by P. Crowcroft, April 1944. Reg. No. W3517 bearing two whole mounts from *Pseudophritis urvillii* from Risdon, Tasmania.
Coitocaecum zealandicum Hine, 1977.	N.M.N.Z. One holotype slide (ZW 1028) and one paratype (ZW 1029) prepared by M. Hine, from Torrent fish, Wellington, N.Z.

Abbreviations are as follows:

T.M.	Tasmanian Museum, Tasmania.
U.S.D.A.	United States Department of Agriculture.
U.S.N.M.H.C.	United States National Museum Helminth Collection, Beltsville, Maryland.
A.M.	Australian Museum, Sydney.
N.M.N.Z.	National Museum of New Zealand, Wellington.

To help elucidate the taxonomic status of the New Zealand form of *C. parvum* and its relationship with the other New Zealand species *C. zealandicum*, measurements were taken of all material collected. Drawings were also made of these specimens for comparison with *C. parvum* from New Zealand.

1.3 RESULTS

1.3.1 Completion of the Life History

(a) Experiment 1

Experiment 1 was designed to complete the life history of *C. parvum* by cycling cercariae of known ancestry through three laboratory hosts. Cercariae derived from naturally infected snails were used to infect 48 laboratory-reared amphipods. These amphipods were fed to three laboratory-raised fish. All three fish were found to contain adult *C. parvum* upon dissection. Of the 11 worms collected, only three had eggs in the uterus. The remaining eight worms were sexually mature but without eggs. Seven eggs in all were collected from ovigerous adults. An attempt was made to infect laboratory-reared snails with miracidia from these eggs but this was not successful.

(b) Experiment 2

Mysids from Timber Yard Point were seen to contain *Coitocaecum* metacercariae. In Experiment 2, these were fed to eight laboratory-raised bullies. Thirty-nine *C. parvum* worms were obtained from the small intestine of seven of the fish. Nineteen of these worms were ovigerous and yielded a total of 47 eggs. These eggs were used to infect laboratory-reared snails. Of the ten snails used in this experiment, one was found to contain sporocysts upon dissection after two weeks. Cercariae

within these sporocysts were immature so positive identification was not possible. However, there was no doubt that they were larval stages of *C. parvum* since the laboratory-reared snails were known to be parasite-free before experimentation. Trout and salmon fry did not become infected with *C. parvum* when fed naturally infected mysids.

(c) Experiment 3

In Experiment 3, 30 parasite-free, field collected mysids were infected with cercariae from naturally infected snails. All mysids were found to be infected with metacercariae one week after infection. Metacercariae were identical to those found in naturally infected mysids and amphipods.

(d) Experiment 4

Bullies collected from Timber Yard Point were found to contain adult *C. parvum*. Seventy-four eggs obtained from these worms were used to infect laboratory-reared snails. One of the 30 snails used in this experiment was found to be shedding microcotylocercous cercariae after four months. The remaining snails were dissected, none of these was infected. Cercariae obtained from this experiment were morphologically identical to those used in Experiment 1. These had previously been collected from naturally infected snails.

The series of four experiments outlined above represent the complete life history of *C. parvum* through laboratory-raised hosts. Adult *C. parvum*, identical to those collected from naturally infected fish, were obtained from Experiments 1 and 2. Sporocysts and cercariae were obtained from experimental infections of snails in Experiments 2 and 4. These cercariae were identical to those found in naturally infected snails. Metacercariae were collected from mysids in Experiment 3. By inference, they were also obtained from amphipods in Experiment 1, for these amphipods were fed live to parasite-free fish. Infections of fish could be derived from no other source than from metacercariae in experimentally infected amphipods.

1.3.2 Field Hosts

(a) Fish hosts

Five of the seven fish species examined were found to be parasitised with *C. parvum*. These were the common bully, *Gobiomorphus cotidianus*; the upland bully, *G. breviceps*; the Inanga, *Galaxias maculatus*; the common smelt, *Retropinna retropinna*; and eels, *Anguilla* spp. The prevalence and intensity of infection in these hosts is listed in Table 1.4.

Only two of the species of fish examined were not infected with *C. parvum*. These were the torrent fish, *Cheimarrichthys forsteri*, and the goldfish, *Carassius auratus*. The torrent fish, *C. forsteri*, was the only species of fish found to be infected with *C. zealandicum*.

(b) Snail host

Only one of the six species of snails sampled was found to be infected with *C. parvum* sporocysts. This was *Potamopyrgus antipodarum*.

(c) Crustacean hosts

Two of the six crustacean species examined were found to be infected with metacercariae of *C. parvum*. These were the mysid *Tenagomysis chiltoni*, and the amphipod *Paracalliope fluviatilis*. Progenetic metacercariae occurred in both of these hosts. The prevalence and intensity of infection with progenetic metacercariae and the mean number of eggs per cyst were recorded over a five month sampling period. As sample sizes were small, the results were pooled and are presented in Table 1.5.

There was a significant difference in the prevalence of *C. parvum* infections in each host type ($\chi^2 = 12.2$, $P < 0.001$). More mysids carried infections of *C. parvum* than amphipods. However, amphipods were more likely to be infected with progenetic metacercariae than mysids ($\chi^2 = 9.3$, $P < 0.01$). There was no significant difference between the intensity of progenetic cysts in amphipods and mysids (Wilcoxon's two sample test, $C = 202.5$, $P > 0.05$). The mean number of eggs per progenetic metacercaria was also significantly different between hosts ($t = 2.88$, $P < 0.05$). Sexually mature metacercariae in amphipods usually contained more eggs than cysts in mysids. The reasons for these differences are not known.

Table 1.4 Definitive fish hosts of *Coitocaecum parvum*.

Species and Common Name	N=	No. Infected	Prevalence (%)	Intensity ($\bar{x}$)	Range
Gobiomorphus cotidianus Common bully	90	84	93	4.3	1-14
Gobiomorphus breviceps Upland bully	15	9	60	3.2	1-6
Galaxias maculatus Inanga	12	8	67	2.4	1-4
Retropinna retropinna Common smelt	57	15	26	1.1	1-3
Anguilla spp. Eels	38	6	17	3.3	1-5

Table 1.5 Comparison between *Tenagomysis chiltoni* and *Paracalliope fluviatilis* as hosts for *Coitocaecum parvum*.

(a) Prevalence of infection.

Host	No. Sampled	Prevalence of infection (%)	Prevalence of infection with progenetic *C. parvum* (%)
T. chiltoni (mysid)	227	54	16.4
P. fluviatilis (amphipod)	143	37	39.2

(b) Intensity of progenetic cysts and mean number of eggs per cyst.

Host	No. Infected Hosts	Total No. Gravid Metacercariae	Intensity of Progenetic Metacercariae ($\bar{x}$)	Mean No. Eggs Per Cyst
T. chiltoni (mysid)	20	30	1.5	26.5
P. fluviatilis (amphipod)	19	22	1.16	99.1

Tenagomysis chiltoni was also found to act as an intermediate host for *Deretrema minutum*. This is a new record and is discussed in Appendix II (page 100).

Four species of crustaceans were not infected with *C. parvum*. These were the mysid *T. macropsis*, the amphipod *Paracorophium lucasi*, the shrimp *Paratya curvirostris*, and the freshwater crayfish *Paranephrops zealandicus*.

1.3.3 Descriptions

(a) Adults

The sources of adults of *C. parvum* used in the descriptions are as follows. Ten adults from the common bully *Gobiomorphus cotidianus* from Taumatu Stream, a further three specimens from experimentally infected laboratory-reared common bullies, and five adults from naturally infected upland bullies, *G. breviceps*, collected from Lake Clearwater. (Canterbury)

(i) Description of *Coitocaecum parvum* (Figs 1.3a, 1.4b, Table 1.6B). Body elongate, ovoid, blunt at anterior end, broadest at or posterior to acetabulum, tapering gradually towards posterior which is rounded. Tegument smooth, aspinous. Oral sucker smaller than acetabulum, oral opening subterminal, ventral, leading to short prepharynx. Pharynx muscular, followed by oesophagus. Caeca simple, thin-walled, extending posteriorly parallel to body wall, uniting within a short distance of hindmost testis. Anal canal, anus absent. Acetabulum large, strongly muscular, covered by numerous micropapillae, ventral, in mid region of body.

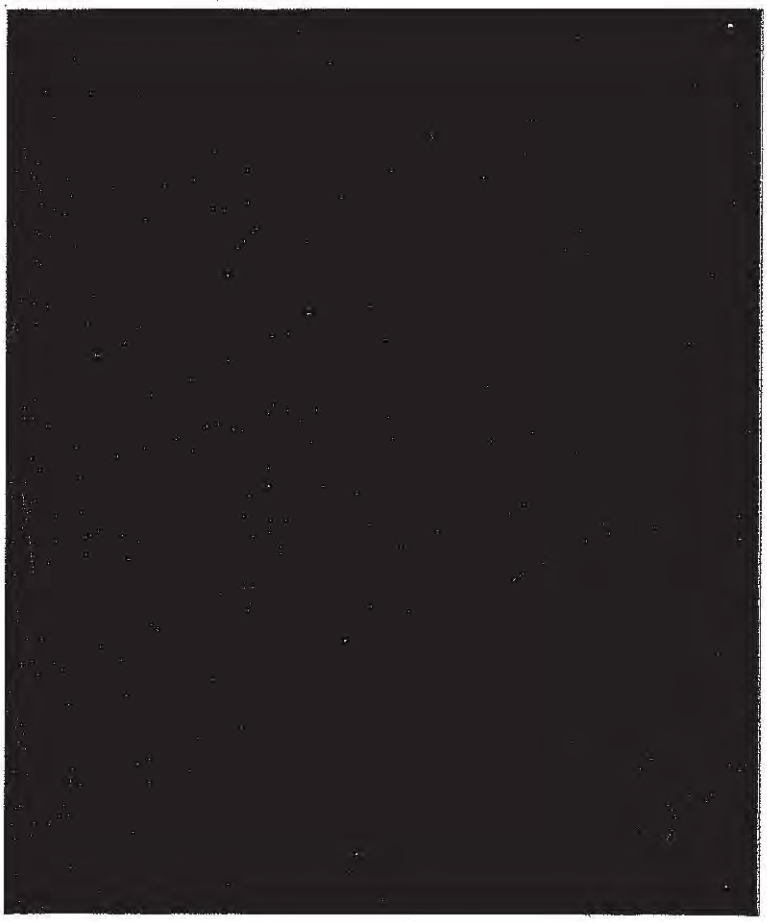
Testes, a pair, ventral, in posterior portion of the body. Anterior testis smaller than posterior, tandem or nearly so. Intercaecal. Sperm duct leaves anterior dorsal edge of each testis, passes dorsally to right of acetabulum to join with the seminal vesicle. Seminal vesicle dorsal, sac-like, to right of acetabulum, frequently reaching posterior edge of acetabulum. Cirrus sac small, ovoid, enclosing terminal portion of seminal vesicle and ejaculatory duct. Duct coils once within cirrus sac, crosses left caecum beneath intestinal fork, joins with uterus to form common duct. Genital pore ventral, left of midline, level or slightly forward of intestinal fork. Prostatic cells present. Cirrus smooth.

Fig. 1.3 Photographs of the seminal vesicle and cirrus sac of

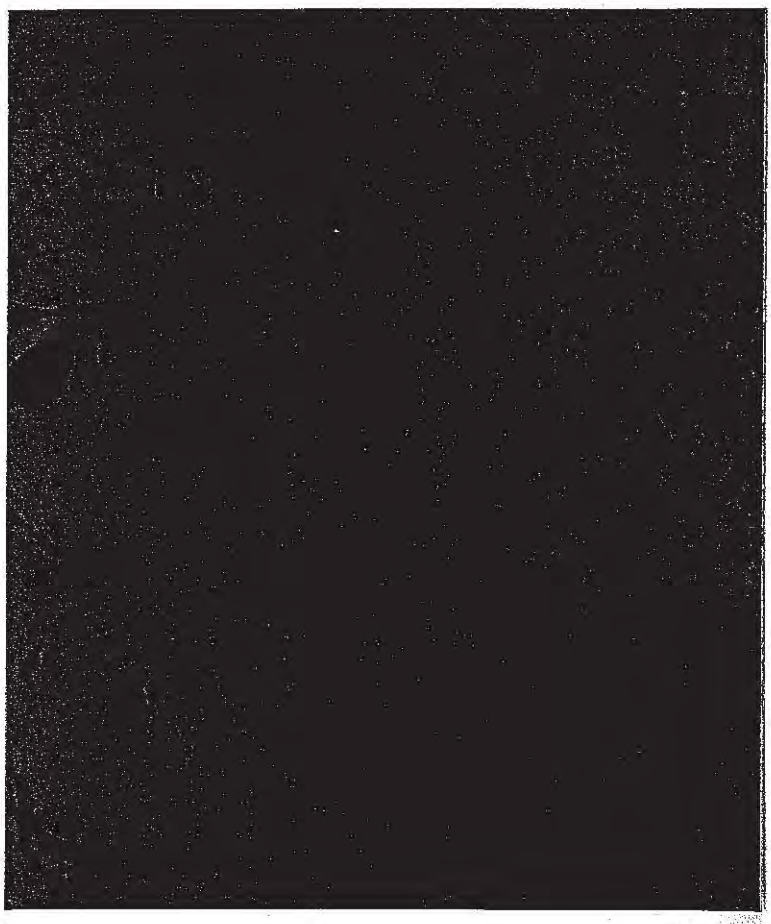
A. *Coitocaecum parvum* (N.Z.)

B. *Coitocaecum anaspidis* Hickman, 1934 (TAS.) syntype. Reg. No. K553.

A = acetabulum; S.V. = seminal vesicle; C.S. = cirrus sac.



A



B

Fig. 1.4 Four species of *Coitocaecum* drawn from type and prepared slides.

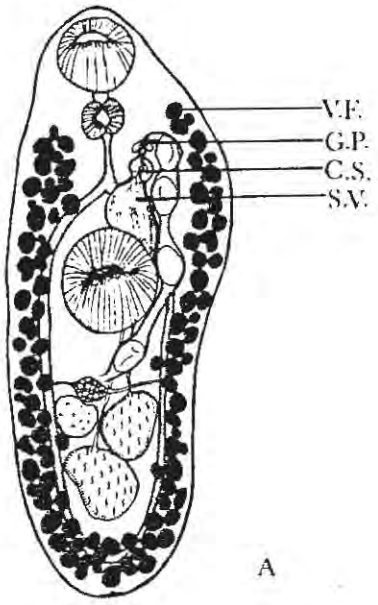
A. *C. parvum* Crowcroft, 1944, ex *Pseudaphritis urvilli*, Loc. Risdon (Tas.). W.3517.

B. *C. parvum* ex *Gobiomorphus cotidianus*, Loc. Canterbury (N.Z.).

C. *C. anaspidis* Hickman, 1944, ex *Anaspides tasmaniae*, Loc. New Town Creek (Tas.). K550.

D. *C. zealandicum* Hine, 1977, ex *Cheimarrichthys forsteri*, Loc. Wellington (N.Z.). ZW1028.

C.S. - cirrus sac; G.P. - genital pore; S.V. - seminal vesicle; V.F. - vitelline follicle.



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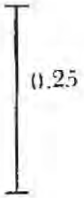

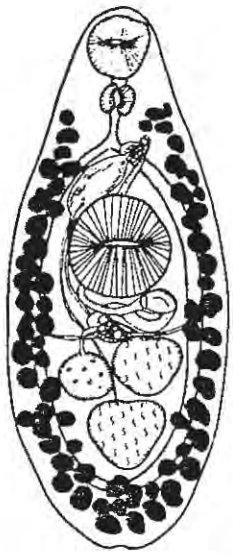

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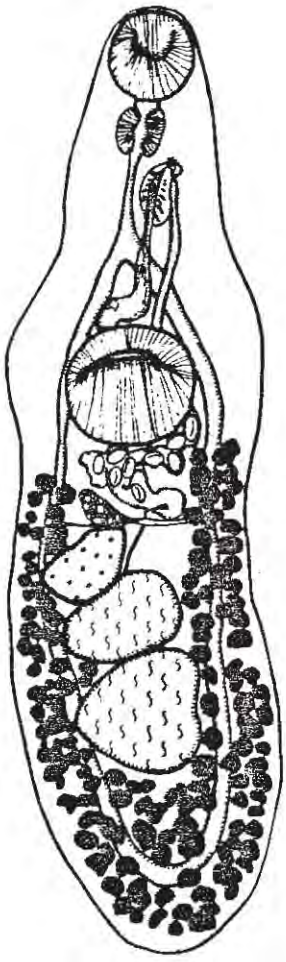

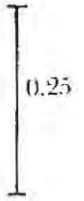

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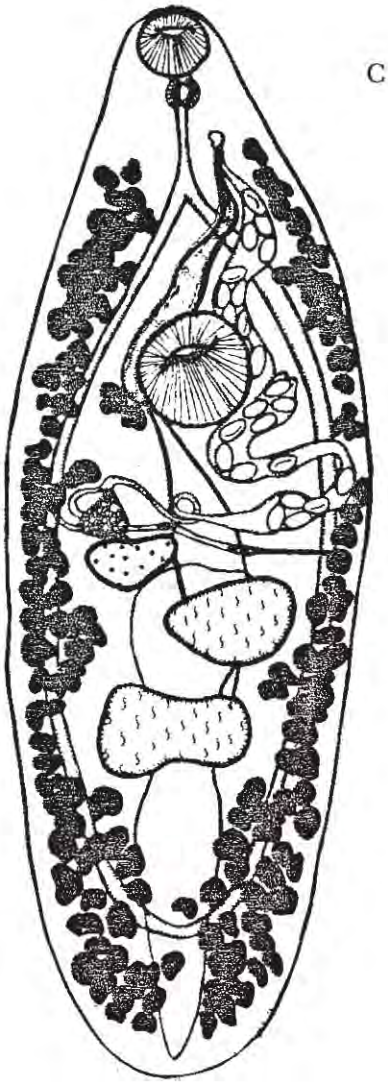


Ovary pear-shaped, smaller than testes, ventral, right of midline, intercaecal posterior to acetabulum. Oviduct leaves antero-dorsal edge of ovary, passes dorsally, receives Laurer's canal and vitelline duct before entering ootype. Uterus leaves ventral side of Mehlis gland, loops upon itself, passes dorsal to acetabulum and seminal vesicle, metraterm joins with ejaculatory duct to form common canal, terminates at genital pore. Uterus contains 3-5 eggs. Eggs ovoid, operculate. Vitelline follicles numerous in most specimens, dorsal, lateral, extending posteriorly to end of body and anteriorly to level of pharynx.

Principal excretory ducts a pair, flame cell formulae 2(2+2+2+2) = 16. Excretory vesicle in posterior portion of body, reaching anteriorly to level of ovary, posteriorly joins with excretory canal to open to outside.

(ii) Other species. Table 1.6 gives the dimensions of four species of *Coitocaecum* - *C. parvum* (New Zealand [B] and Tasmanian forms [A]), *C. anaspidis* [C], and *C. zealandicum* [D]. Drawings and measurements were taken from syntypes, co-types and holotypes of borrowed specimens.

(b) Eggs and miracidia (Fig. 1.5a, b)

Eggs and miracidia used in descriptive work were collected from adult *C. parvum* taken from naturally infected bullies. Eggs were also collected from progenetic metacercariae for comparison with eggs from adult worms.

(i) Eggs. Thirty-five freshly laid eggs from adult worms measured 0.072 × 0.039 mm (0.069 - 0.083 × 0.034 - 0.045 mm). The eggs were tanned, ovoid and operculate. The operculum measured 0.016 mm (0.013 - 0.020 mm) in diameter. Eggs were usually slightly asymmetrical about the long axis. Freshly laid eggs were undifferentiated.

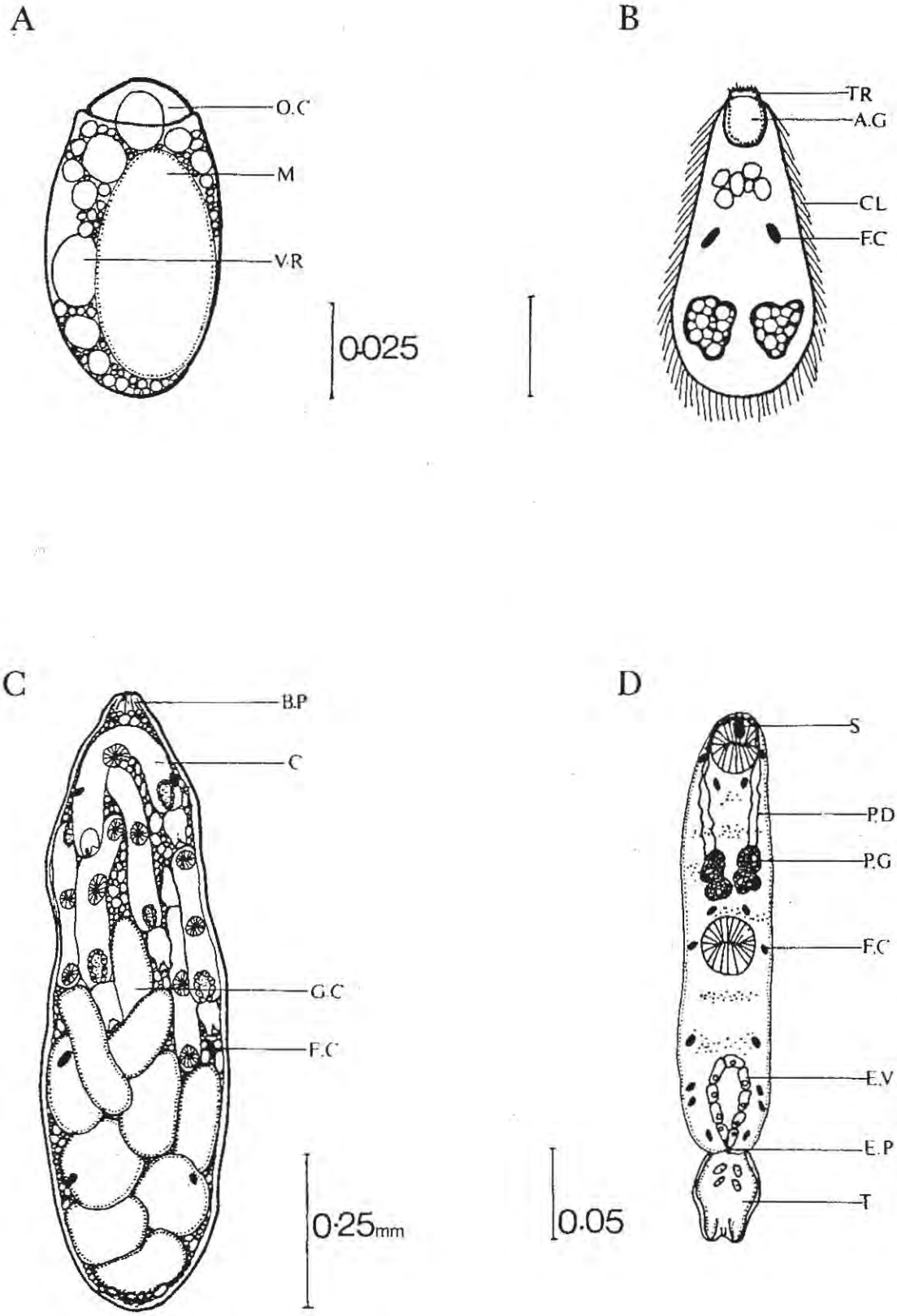
Freshly laid eggs from progenetic metacercariae were identical to the eggs described above. However, eggs collected from the cavity of metacercarial cysts were much more variable in their dimensions. The smallest measured 0.048 × 0.025 mm. Often small tanned concretions could be found along with eggs in the cyst cavities. It is likely that these were produced by metacercariae at the onset to egg production proper.

Table 1.6 Dimensions of three species of *Coitocaecum* - *C. parvum* Crowcroft, 1944 (Tasmania); *C. parvum* (New Zealand); *C. anaspidis* Hickman, 1934; and *C. zealandicum* Hine, 1977. (All measurements in mm.)

	A *C. parvum* (Tas.) N = 2	B *C. parvum* (N.Z.) N = 18 ±S.D.	C *C. anaspidis* N = 4	D *C. zealandicum* N = 2
Body length	0.75	0.80±0.12	2.2(2.0-2.9)	2.1
breadth	0.29	0.30±0.08	0.52(0.5-0.53)	0.51
Oral sucker	0.09×0.09	0.12±0.03×0.11±0.016	0.16×0.15	0.19×0.19
Prepharynx	0.02	0.024±0.012	0.041×0.061	0.036×0.053
Pharynx	0.43×0.53	0.054±0.013×0.06±0.008	0.073×0.061	0.12×0.09
Oesophagus length	0.04	0.045	0.17	0.19
Ventral sucker	0.16×0.14	0.19±0.033×0.18±0.028	0.27×0.27	0.34×0.27
Anterior testis	0.12×0.10	0.15±0.027×0.12±0.016	0.26×0.16	0.29×0.21
Posterior testis	0.18×0.15	0.16±0.039×0.13±0.018	0.29×0.17	0.29×0.27
Cirrus sac	0.03×0.01	0.046×0.037	0.08×0.02	0.08×0.05
condition	ovoid	ovoid	long	ovoid
Seminal vesicle	0.12×0.06	0.14±0.03×0.06±0.011	0.38×0.07	0.21×0.073
Ovary	0.07×0.06	0.13±0.02×0.12±0.03	0.17×0.14	0.22×0.13
Egg reservoir	0.07×0.05	0.06×0.05	0.15×0.07	0.13×0.05
Vitelline follicles	0.02-0.038	0.05±0.01×0.04±0.01	0.07×0.06	0.04×0.03
Eggs	0.061-0.067×0.038-0.058	0.079±0.006×0.44±0.01	0.061-0.092×0.032-0.048	0.073×0.043
Prostate gland	present	present	?	?
Pars prostatica	indistinct	present	?	present

Fig. 1.5 Larval stages of *Coitocaecum parvum*. A - egg; B - miracidium; C - sporocyst; D - microcotylocercous cercaria.

A.G - apical gland; B.P - birth pore; C - cercaria; CL - cilia; E.P - excretory pore; E.V - excretory vesicle; F.C - flame cell; G.C - germinal cell; M - miracidium; O.C - operculum cap; P.D - penetration duct; P.G - penetration gland; S - stylet; T - tail; TR - terebratorium; V.R - vitelline reminant.


A
O.C
M
V.R
0·025
B
TR
A.G
C.L
F.C
C
B.P
C
G.C
F.C
0·25mm
D
S
P.D
P.G
F.C
E.V
E.P
T
0.05

(For further discussion on eggs from progenetic metacercariae, see Chapter 2, page 54.)

(ii) Miracidia description (Fig. 1.5a). Ten miracidia examined live ranged in size from 0.069 × 0.032 to 0.086 × 0.053 mm. The body was pear-shaped, widest at anterior, tapering to posterior. Body entirely ciliated, except for region of the terebratorium. Short ciliated hairs surrounded the outer edge of this organ. Apical gland, single, median, directly beneath apical region. Contents of the gland were coarsely granular. Eyespots absent. A pair of flame cells present in posterior portion of body.

(c) Sporocysts (Fig. 1.5c)

Sporocysts used in descriptive work were collected from naturally and experimentally infected snails.

Two mother sporocysts collected from experimentally infected snails were colourless and transparent measuring 0.54 × 0.12 and 0.48 × 0.10 mm respectively. No birth pore has been seen. The lumen of the mother sporocysts contained germinal cells (0.084 × 0.052 mm) at different stages of development but daughter sporocysts were not observed. Both mother sporocysts were found attached to the viscera of the infected snail.

Fifteen daughter sporocysts of *C. parvum* collected from naturally infected snails measured 1.15 ± 0.31 mm in length (Fig. 1.5c). Sporocysts were found in the digestive gland and gonads of the host. The number of sporocysts per snail varied between 7 and 13. MacFarlane (1939) has previously described the sporocysts of *C. parvum* and therefore this shall not be repeated.

(d) Cercariae (Fig. 1.5d, Table 1.7)

Cercariae used in descriptions were collected as they emerged from experimentally and naturally infected snails.

(i) Description (Fig. 1.5d, Table 1.7). Body elongate, narrow, blunt at both ends, broadest at level of acetabulum. Tegument with numerous micropapillae, aspinous. Tail short, cup-shaped is concave at

Table 1.7 Dimensions of cercariae of *Coitocaecum parvum* (N = 10).

	Mean (mm)	~~Range~~ (mm) S.D.
Total length	0.34	±0.08
Width	0.058	±0.009
Body length	0.29	±0.035
Tail length	0.053	±0.0072
Tail width	0.039	±0.0012
Oral sucker (length x width)	0.046 x 0.039	±0.0023 x ±0.0031
Acetabulum	0.041 x 0.043	±0.0015 x ±0.0011
Excretory vesicle	0.064 x 0.037	±0.0032 x ±0.0011
Stylet	0.025 - 0.012	-

posterior to form sucker. Oral sucker equal to acetabulum, ventral, covered with micropapillae, subterminal. Distinctive stylet, two-pronged, on anterior edge of oral sucker. Stylet shaft with double apical spike, also two small lateral spikes. No digestive system observed. Three pairs of penetration glands present slightly anterior to acetabulum. Filled with granular material. Ducts run dorsal, anteriorly to openings above level of oral sucker.

Acetabulum papillate, not strongly muscular as in adult, ventral, lies in posterior portion of the body. Excretory ducts arise anteriorly, run latero-posteriorly to join excretory vesicle. Flame cell formula as in adult, $2(2+2+2+2) = 16$.

(e) Metacercariae (Fig. 1.6, Table 1.8)

Metacercariae used in descriptions were collected from experimentally and naturally infected mysids and amphipods.

Coitocaecum parvum metacercariae collected two hours after encystment measured 0.27 ± 0.003 by 0.054 ± 0.0015 mm. They were morphologically identical to cercariae apart from the absence of the tail and presence of a cyst wall. These metacercariae formed small cysts which measured 0.12 ± 0.0013 by 0.14 ± 0.012 mm.

Metacercariae grew considerably after encystment (Fig. 1.6). Immature metacercariae only differed from ovigerous metacercariae in the size of major organs, degree of development of the reproductive system, and the absence of vitelline follicles (Table 1.8).

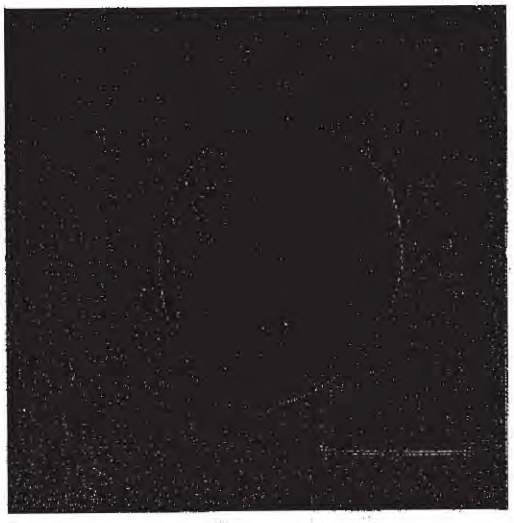
Ovigerous metacercariae were identical to adults from experimentally infected fish hosts, therefore a description shall not be repeated.

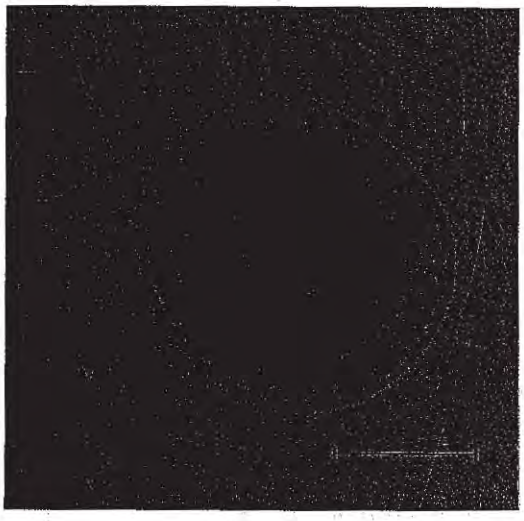
1.4 DISCUSSION

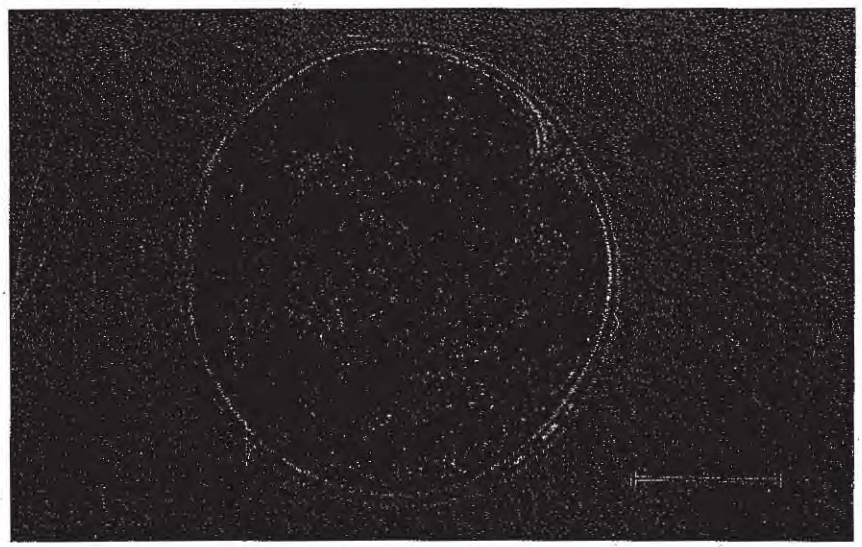
1.4.1 Identification of the New Zealand *Coitocaecum*

There are two problems encountered in identifying adult trematodes solely by their morphology and dimensions. The first is a lack of uniformity in the method of treatment and fixation of specimens (Ulmer, 1950;

Fig. 1.6 Photograph of *Coitocaecum parvum* metacercariae showing changes in cyst size due to growth of metacercariae. Cysts vary from newly encysted to progenetic egg-bearing metacercariae. (Plate I) Ex *Tenagomysis chiltoni*. Loc. Lake Ellesmere (N.Z.)







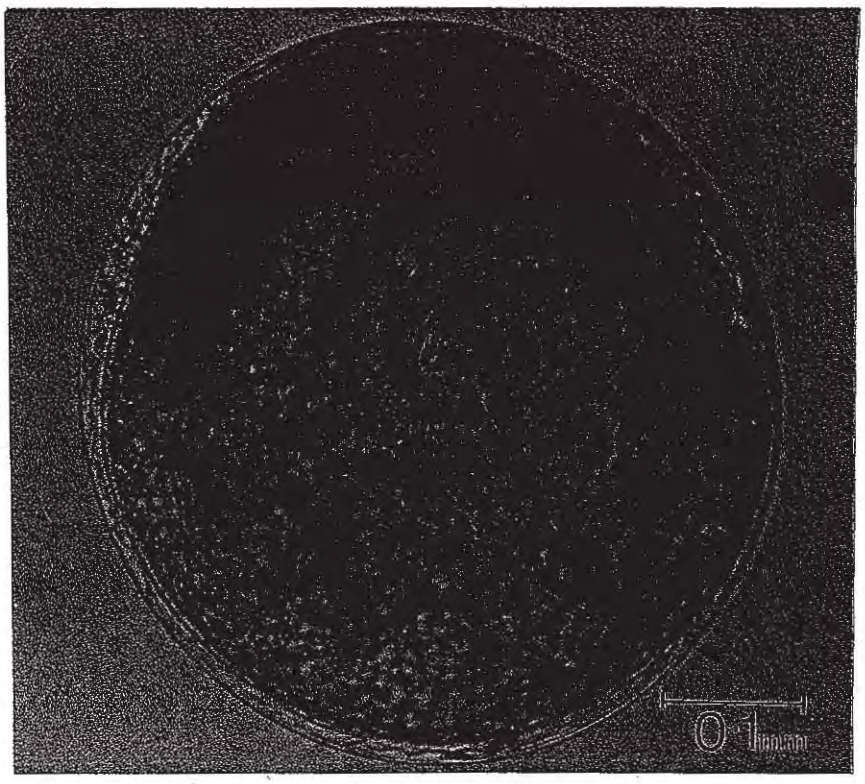


Table 1.8 Dimensions of *Coitocaecum parvum* metacercariae, ovigerous and immature (without eggs).

	Ovigerous (mm) N = 10	Immature (mm) N = 21
Body length	0.78 ± 0.18	0.32 ± 0.26
breadth	0.31 ± 0.11	0.17 ± 0.15
Oral sucker	0.13 ± 0.03 x 0.11 ± 0.013	0.07 ± 0.05 x 0.06 ± 0.05
Prepharynx	0.021 ± 0.015	0.012 ± 0.041
Pharynx	0.032 ± 0.021 x 0.04 ± 0.01	0.031 ± 0.003 x 0.01 ± 0.007
Oesophagus length	0.047	0.021 ± 0.0017
Ventral sucker	0.16 ± 0.036 x 0.18 ± 0.032	0.07 ± 0.003 x 0.06 ± 0.004
Anterior testis	0.16 ± 0.024 x 0.15 ± 0.104	0.09 ± 0.002 x 0.08 ± 0.001
Posterior testis	0.17 ± 0.041 x 0.16 ± 0.017	0.09 ± 0.002 x 0.06 ± 0.002
Cirrus sac	0.039 x 0.036	-
condition	ovoid	-
Seminal vesicle	0.13 ± 0.04 x 0.05 ± 0.13	-
Ovary	0.14 ± 0.019 x 0.13 ± 0.021	0.06 ± 0.0.014 x 0.07 ± 0.0.001
Egg reservoir	0.05 x 0.05	-
Vitelline follicles	0.06 ± 0.01 x 0.04 ± 0.013	-
Eggs	0.076 ± 0.005 x 0.39 ± 0.021	-
Prostate gland	present	-
Pars prostatica	present	-

Sinclair, 1971). Watson (1981) found significant changes in the measurements of *Metorchis conjunctus* after dehydration of the adult worms in alcohol. The second problem is that of individual diversity between adults of the same species (Stunkard, 1957; Blackwelder, 1967; Mayr, 1969; Cheng, 1973; MacKenzie and McKenzie, 1980). Betterton (1980), for example, found considerable variation in the body shape, vitelline fields and morphology of *Euparadistomum buckleyi* and *E. pearsoni* from seven species of mammal definitive hosts.

Both of these problems are taken into account during identification of trematodes in this study. Where possible, large numbers of adult flukes have been examined. Characters used in identification of species included those considered least likely to show morphological variation. These are the form of the cirrus sac, arrangement of the male and female reproductive systems, and extent of the vitellaria in relation to the genital pore. Characters used secondarily in identification are the dimension of major organs, egg size, and position of the reproductive organs.

Since MacFarlane (1939) reported *C. anaspidis* Hickman, 1934, in New Zealand this species name has become firmly entrenched in the literature and is referred to by many authors (Buttner, 1951; Manter, 1954; Dix, 1968; Livingston, 1970; Hewitt and Hine, 1972; Erasmus, 1972; Hine, 1977; McDowall, 1978; Hine and Francis, 1980). However, Crowcroft (1944), in a critical examination of the species descriptions of Hickman (1934) and MacFarlane (1939), cast some doubt on the identity of the New Zealand species.

Crowcroft (1944) wrote:

> "In view of the differences between the original description of the species, and MacFarlane's account of the New Zealand form, the identity of the latter must be in doubt until material from both sources is available to the one investigator."

Examination of the type material of *C. anaspidis* Hickman, 1934, adults described as *C. anaspidis* from eels in New Zealand (Manter, 1954), and adults obtained from experiment and from natural infections has revealed several differences between the Tasmanian and New Zealand specimens. These differences were as follows.

(1) The vitellaria (Fig. 1.4c) in *C. anaspidis* extends into the neck region, anterior to the intestinal fork but well behind the level of the pharynx. The genital pore opens level with the foremost vitelline follicle. In the New Zealand *Coitocaecum* the vitellaria extends well into the neck region and ends level with the pharynx (Fig. 1.4b).

(2) In *C. anaspidis* the seminal vesicle is long and thin and almost entirely enclosed along its length by the cirrus sac (Fig. 1.3b). The ejaculatory duct coils several times within the terminal portion of the cirrus sac. In the New Zealand form the seminal vesicle is short and fat, the ejaculatory duct coils once within the cirrus sac. The cirrus sac is small and distinctly ovoid (Fig. 1.3a).

(3) The ovary in *C. anaspidis* is spherical and gives rise to the oviduct anteriorly. The Laurer's canal runs across the body loops upon itself and opens to a dorsal pore situated just to the left of the mid-line. In the New Zealand species the ovary is pear-shaped and leads to the oviduct anteriorly. However, the Laurer's canal runs directly across to the dorsal pore well to the left of the body.

(4) There are also striking differences between the size of *C. anaspidis* and the New Zealand specimens. Figure 1.4 is drawn to scale and shows the difference in size between the specimens preserved by Hickman (1934) and the typical size of adults examined in this study. Although Hickman's type slides were well flattened, adults of this size have never been observed from New Zealand hosts.

MacFarlane (1936, 1939) did not discuss the differences between his specimens and Hickman's description of progenetic *C. anaspidis*. However, in reference to the dimensions of *C. anaspidis*, MacFarlane wrote, "Average excludes Hickman's figures which are taken from the large progenetic individuals and are thus greater than those of a normal adult parasite." (page 174). It should be pointed out that Wisniewski (1933) and Dollfus (1959) found no significant differences between progenetic metacercariae of *Coitocaecum testiobliquum* and of *Nicolla gallica* and adults of these species. In addition, no significant differences were found between progenetic metacercariae from naturally infected amphipods and mysids, and adults from fish hosts examined in this study.

Of the 28 species of *Coitocaecum* described to date, the adult derived from experimental infections of fish resembles most closely *C. parvum* Crowcroft, 1944, from Tasmania. Dollfus (1959) considered *C. parvum* to be a synonym of *C. anaspidis* although his reasons for this are unclear. Similarities between the two forms include the extent of the vitellaria, position of the genital pore, structure of the male and female reproductive systems, and size. In addition, there are similarities in the definitive fish hosts. *Coitocaecum parvum* from Tasmania was described from *Galaxias attenuatus* (=*G. maculatus*), and *Pseudaphritis urvillii*. The New Zealand form of *C. parvum* is also found in the Inanga, *Galaxias maculatus*.

However, there are some differences between these two forms. In *C. parvum* (Tasmania) the seminal vesicle and uterus lie close to the dorsal surface to the *right* of the mid-line. They cross the left branch of the intestinal caecum and terminate at the genital pore (Fig. 1.4a). The acetabulum of *C. parvum* (Tasmania) is also described as possessing papillae although Crowcroft (1951) noted that these were not always present. In *C. parvum* (New Zealand) the inner gape of the acetabulum is usually smooth. However, on rare occasions a few specimens have been found with papillae similar to those seen on the type slides of *C. parvum* (Tasmania). This condition is not typical in *C. parvum* (New Zealand). The differences between *C. parvum* from New Zealand and Tasmania are not, at present, considered enough to separate the two as distinct species. Crowcroft (1951) examined over 60 whole mounts of *C. parvum* and noted considerable variation in the form and placement of major organs in these specimens. Such variations included displacement of position and variable size of the seminal vesicle, absence of one or both testes, and separation of the intestinal caeca.

Considering the natural diversity of form that occurs within a species (Stunkard, 1957; Mayr, 1969; Futuyma, 1979), it is likely that the New Zealand specimens are closely related to *C. parvum* from Tasmania. At present, there are insufficient differences to distinguish them as distinct species.

The two species of *Coitocaecum* - *C. parvum* and *C. zealandicum* - found in the present study from Canterbury fishes, are clearly distinct in appearance. The most obvious difference between the two species is the extent of the vitelline fields. In *C. zealandicum* the vitellaria are entirely post-acetabular (Fig. 1.4d). In addition, the uterus crosses the

left side of the body, unlike in *C. parvum* where it crosses the right. *Coitocaecum zealandicum* is considerably larger than *C. parvum* and has only ever been recorded from the Torrent fish.

1.4.2 Life History of *Coitocaecum parvum* in New Zealand

MacFarlane (1939) first proposed the life history of *C. parvum* (under the name *C. anaspidis*) in New Zealand based on similarities between larval stages taken from the field and from infection of tank bred *Gobiomorphus gobiodes* (Giant bully). In this chapter, the life history of *C. parvum* has been confirmed under laboratory conditions.

The life history stages of *C. parvum* derived from experimental infections of laboratory hosts were similar in morphology to those described by MacFarlane (1939). However, some minor differences were found between adults described by MacFarlane and those examined in this study. These were as follows:

(1) The cirrus sac in *C. parvum* was short, distinctly ovoid, and enclosed the terminal portion of the ejaculatory duct. MacFarlane did not describe the form of the cirrus sac in his specimens but in his figures it appeared as long and thin.

(2) MacFarlane described the oviduct as leaving the ovary laterally. In this study, the oviduct was observed leaving the ovary antero-dorsally.

(3) In *C. parvum* the metraterm of the uterus and the cirrus form a common canal before entering the genital pore. MacFarlane described them as opening side by side at the genital pore.

Some differences have also been found between *C. parvum* metacercariae examined in this study and those examined by MacFarlane.

MacFarlane noted that progenetic metacercariae infecting *Paracalliope fluviatilis* do not have sperm in the testes, seminal vesicle or seminal receptacle even though well formed eggs were present. He concluded that the undeveloped condition of the testes made fertilisation impossible, and suggested that miracidia had most likely arisen by parthenogenesis. In

this study I had the opportunity to examine large numbers of live ovigerous metacercariae from both mysid and amphipods. On numerous occasions active spermatozoa were seen to fill the seminal vesicle, and sometimes the seminal receptacle. In all cases, metacercariae were enclosed within the cyst membrane, making cross-fertilisation impossible. It is therefore suggested that metacercariae at least have the potential for self-fertilisation.

The production of eggs by encysted metacercariae raises some interesting questions concerning a possible abbreviation of the three-host life history. These questions will be examined in the following chapter.

1.5 SUMMARY

In this chapter the identity and life history of *Coitocaecum parvum* Crowcroft, 1944, has been determined by comparisons with other members of the genus and by experimental infections of laboratory-reared hosts.

Coitocaecum parvum Crowcroft, 1944, from Canterbury utilises several hosts in its life history. Adults are found in several freshwater fish species, *Gobiomorphus cotidianus, G. gobiodes, G. breviceps, Retropinna retropinna, Galaxias maculatus* and *Anguilla* spp. Eggs pass out in the faeces of the fish host and continue development in freshwater. Miracidia hatch from these eggs and infect the mollusc *Potamopyrgus antipodarum*. Sporocysts developing in this host release microcotylocercous cercariae which are free-swimming and short-lived. Upon location of a suitable second intermediate host, either the mysid *Tenagomysis chiltoni* or the amphipod *Paracalliope fluviatilis*, cercariae penetrate, encyst, and continue development. Fish become infected with *C. parvum* by ingesting infected crustaceans.

CHAPTER II

AN ABBREVIATED LIFE HISTORY IN *COITOCAECUM PARVUM*

2.1 INTRODUCTION

As indicated in the previous chapter, a small proportion of *Coitocaecum parvum* metacercariae exhibit progenesis while encysted in the second intermediate hosts, *Tenagomysis chiltoni* and *Paracalliope fluviatilis*. Progenesis often results in the production of numerous eggs which accumulate within the cavity of the cyst. As a consequence of this egg production, the possibility exists for an abbreviation of the life history of *C. parvum* in which only two hosts may be utilised instead of the normal three. In order for such an abbreviation to occur, eggs from progenetic metacercariae, presumably resulting from self-fertilisation, must be capable of hatching and infecting the first intermediate host, *Potamopyrgus antipodarum*. In addition, some mechanism must be available to allow the dispersion of eggs from their enclosing cyst wall into the surrounding environment.

The purpose of this chapter is to test whether or not an abbreviated life history is possible in *C. parvum*.

The significance of progenesis in the life histories of trematodes has been discussed by many. Some, like La Rue (1951), Stunkard (1959), and Yamaguti (1971) regard sexual maturity of trematodes in an intermediate host as a relict of a primitive two-host, mollusc-invertebrate cycle, with little scope for further evolutionary advance. This theory has not been widely accepted. Others such as Buttner (1950, 1952), Pearson (1972), Grabda-Kazubska (1976), and Font (1980) considered it to be a more recent appearance in the life histories of trematodes. Pearson (1972) argued that the most primitive Digenean life history probably involved only a mollusc and vertebrate (e.g., sanguinicolids, spirorchids, and schistosomes). Later, the metacercarial stage arose which prolonged the life span of the short-lived cercariae by encysting in either an intermediate host or on vegetation. More recently, progenesis or 'early' maturation of metacercariae has evolved resulting in the potential or actual loss of the definitive host. A reduction in the number of hosts required to complete the life history of a trematode is now considered one of the major advantages of progenesis. Not only does a parasite benefit

from elimination of the risk of transferring a metacercaria to the vertebrate definitive host (Font, 1980) but also from an increase in the number of individuals in a parasite population which have the potential to produce eggs (Grabda-Kazubska, 1976).

Although many authors assume that an abbreviated life history follows as a consequence of egg production by metacercariae (e.g., McMullen, 1938; Uzmann, 1953; Srivastava and Ghosh, 1969; Deblock, 1977; Winstead and Couch, 1981), only a few have attempted to test this experimentally. For example, Stunkard (1959) experimentally infected laboratory-reared snails (*Amnicola limosa*) with miracidia from *Asymphylodora* sp. Sporocysts and rediae were found in this host after one week. Wootton (1957) used eggs of *Allocreadium alloneotenicum* from caddis fly larvae to experimentally infect a suitable snail host. Bayanov (1975) found that ova from progenetic metacercariae of *Prosotocus confusus* did not produce miracidia when kept in water, but somehow infected the snail *Bithynia tentaculata* in the laboratory. Other successful infections of snails by miracidia from progenetic metacercariae have been achieved by Dollfus (1938) and Buttner (1950, 1951, 1955). However, in some species, eggs produced by metacercariae were not fertile. Cheng (1957) found the eggs of *Crepidostomum cornutum* from the crayfish *Cambarus bartoni sciotensis* to be infertile and incapable of infecting a molluscan host.

Abbreviation of the life history of *Coitocaecum parvum* is dependent on the viability of eggs from progenetic metacercariae. Three experiments were conducted; in the first, the development and hatching success of eggs was determined. Comparisons were made between freshly laid eggs from progenetic trematodes collected from mysids, amphipods, and from "normal" adults in fish hosts. A second experiment was designed to determine if prolonged storage in cysts affected eggs. Observations were made on the hatching success of eggs collected from the metacercarial cysts. In the third experiment, the ability of miracidia to successfully infect the snail host *Potamopyrgus antipodarum* was tested.

In addition, the problem of egg dispersal from the second intermediate host was examined. Two barriers exist that prevent effective egg dispersal from the intermediate host:

(1) Metacercariae of many species occur within the tissues or haemocoel of their hosts (McMullen, 1938; Wootton, 1957; Jamieson, 1966a; Deblock, 1977; Font, 1980; Winstead and Couch, 1981). Unlike

adult trematodes, which usually occur in the intestines of hosts, there is no obvious exit for eggs from the host tissues.

(2) The metacercarial cyst wall, which is present in virtually all cases and may be thickened, may also act as a barrier to dispersal of eggs.

Many authors have considered egg dispersal to be achieved either when the host is eaten by a predator or when the intermediate host dies and decomposes. However, the latter method does not adequately explain the release of eggs from the confines of the cyst wall.

Hickman (1934) suggested that eggs of *C. anaspidis* were liberated from the cyst by bursting of the cyst wall. Pearson (1972) noted that, as metacercariae increase in size, considerable pressures may be exerted on this structure. This is especially so in cases where the cyst wall is thin. The rupture of the metacercarial cyst wall in response to chemical stimulation is well documented, although all of these reports are for trematodes that have a warm-blooded definitive host (e.g., Howell, 1968; Macy *et al.*, 1968; Erasmus, 1972; Fried and Grigo, 1975, Fried and Butler, 1978; Mohandas and Nadakal, 1978; Fried and Butler, 1979, Radlett, 1979). In many cases experimental excystation involves complicated treatment of the cysts with various digestive enzymes from the definitive hosts combined with high environmental temperatures. Preliminary observations on *C. parvum* metacercariae showed that excystation of cysts occurred on natural death of the second intermediate crustacean hosts. It appeared that chemical factors derived from the autolysing host triggered excystation of *C. parvum* metacercariae. This has been partially investigated in this study.

2.2 METHODS

2.2.1 Viability of Eggs and Infection of Snails

Section I deals with the hatching success and viability of freshly laid eggs from progenetic metacercariae and from adult flukes from fish hosts. The effects of prolonged storage on aged eggs taken from the

cyst cavity of progenetic metacercariae were also examined. Freshly laid eggs were collected as they emerged from the uterus of flukes (either from adults or progenetic metacercariae), while aged eggs, already present within the cysts of progenetic metacercariae on collection, were recovered immediately after the rupture of the cysts. Comparisons were made between the rates and percentage hatch of freshly laid eggs from progenetic metacercariae and from adult flukes, and between freshly laid and aged eggs. In addition, attempts were made to infect laboratory-bred snails with eggs from both progenetic metacercariae and adult flukes from fish hosts.

(a) Sources of eggs

Ten encysted progenetic metacercariae were recovered from naturally infected mysids previously collected from the Selwyn River. Cysts were teased from the tissues of mysids and transferred into a watch glass containing saline (0.75% NaCl in distilled water). The cyst wall of each metacercaria was ruptured by gently squeezing the cyst with a pair of fine forceps. Aged eggs which were already present within the cyst cavity were transferred to distilled water for use in Experiment 2. The progenetic metacercariae, from which these eggs had come, were kept in saline for a further 24 hours. After this time, most of the eggs contained within the uterus had been passed out into the saline. These eggs were collected and transferred to a watch glass containing distilled water for use in Experiment 1.

A further ten progenetic metacercariae were recovered from naturally infected amphipods also collected from the Selwyn River. The method of obtaining freshly laid and aged eggs is outlined above.

An additional ten adult *C. parvum* were taken from two naturally infected bullies (*Gobiomorphus cotidianus*) also collected from the Selwyn River. Fish were killed by decapitation and the intestine removed to a dish containing saline. The intestine was slit lengthwise and adult flukes removed from its wall. Flukes were transferred to a watch glass containing saline where they remained for 24 hours. After this time, eggs that had been laid by the adult worms were removed to a watch glass of distilled water for use in Experiment 1.

The number of eggs obtained from each of the three sources is given in Table 2.1.

Table 2.1 Numbers of eggs collected from three hosts.

Number of Eggs:	Host		
	Mysid	Amphipod	Bullies
Freshly laid	25	32	48
Aged	136	153	-

Experiment 1: Three watch glasses, each containing eggs from progenetic metacercariae from either *Paracalliope fluviatilis* or *Tenagomysis chiltoni* or from adults taken from the fish host *Gobiomorphus cotidianus*, were maintained at room temperature (18-22°C) for three weeks. During this time the water in the watch glasses was changed at two day intervals to ensure a high oxygen content for continued development of eggs. Each day the eggs were examined with a binocular microscope and the number of empty eggs with open opercula (= hatched eggs) was counted and the results recorded. Miracidia, although frequently seen swimming in the water, were not counted as they were found to survive for only a few hours, making counts at 24 hour intervals inaccurate. After three weeks the remaining eggs which showed no signs of development were classified as dead and their numbers recorded.

Experiment 2: One hundred and thirty-six aged eggs were collected from the cysts of progenetic metacercariae from mysids and placed in a Petri dish containing distilled water. A further 153 eggs from progenetic metacercariae from amphipods were also treated in a similar manner. The water was changed at two day intervals to maintain a high oxygen content and left at room temperature (18-22°C). Every day for three weeks the number of eggs which had hatched was recorded. At the end of the experiment the number of dead eggs showing no signs of development was also recorded. The rate and percentage of eggs hatching over the three weeks were determined and the results compared to those in Experiment 1.

Experiment 3: This experiment was conducted to determine whether

or not miracidia from the eggs of progenetic metacercariae were capable of infecting the snail host, *Potamopyrgus antipodarum*. One hundred and twenty snails used in this experiment were collected from a bucket where they had been maintained and allowed to breed for the previous 18 months (see page 10). Sixty of these snails were immediately dissected as controls for this experiment and examined for the presence of larval trematodes. None were found. The remaining 60 snails were divided into three groups each containing 20 individuals and placed in three Petri dishes labelled 1 to 3.

Next, 52 eggs were collected from the cysts of five progenetic metacercariae previously taken from naturally infected mysids. These were added to Petri dish 1. A further 57 eggs were obtained from four metacercarial cysts found in amphipods and added to Petri dish 2.

Eggs used as a control in this experiment were collected from the faeces of naturally infected fish. The procedure for collecting eggs is outlined on page 13 (Chapter I). Fish were killed after their faeces had been collected to ensure that eggs used in this experiment were from *C. parvum*. Only *C. parvum* was present in the intestine of fish examined.

Fifty-two eggs collected from the faeces of fish were placed in Petri dish 3.

Washed *Elodea* and chalk fragments were added to each of the three Petri dishes. After the dishes had been maintained at 18-22°C for two weeks, the water in each dish was examined under a binocular microscope for the presence of *C. parvum* cercariae. Following this, each snail was placed in saline and gently crushed using a pair of fine forceps. The tissues were teased out and examined for the presence of sporocysts. The number of infected snails in each of the three Petri dishes was recorded.

2.2.2 Excystation of Metacercariae

In this section the method of dispersal of eggs from progenetic metacercariae is examined. Preliminary studies on *C. parvum* had indicated that metacercariae from *Tenagomysis chiltoni* excyst soon after death of this host. Two experiments were designed to examine the conditions under which excystation of *C. parvum* metacercariae occurs. Experiment 4 was conducted to determine if excystation of metacercariae took place within

tissues of autolysing mysids. In Experiment 5 tests were made to determine if metacercariae would excyst *in vitro*. In this experiment, the fluid from the hepato-pancreas of mysids was used to stimulate excystation.

Experiment 4: Twenty large mysids were decapitated and placed in groups of five in four Petri dishes containing pond water. After 24 hours each mysid was removed to a watch glass under a binocular microscope and examined for parasites. These were counted and the number which had excysted recorded.

Controls for this experiment consisted of 30 encysted metacercariae which had previously been removed from mysids and maintained in saline for 24 hours. These were examined regularly for signs of excystation.

This experiment was conducted at room temperature (18-22°C).

Experiment 5: Sixty metacercarial cysts were collected from naturally infected mysids. Immediately after removal from the mysids each cyst was washed by transferring them through three baths of saline. Cysts were kept in the third bath of saline for 24 hours to ensure that metacercariae to be used in the experiments were still encysted.

Metacercarial cysts were divided into two groups, 30 to be used as controls and a further 30 as the test group.

Immediately before experimentation, a solution of mysid host enzymes was made by removing the hepato-pancreas from 20 freshly killed mysids. The hepato-pancreas was homogenised in 0.5 ml of distilled water. Five drops of this fluid were added to the watch glass containing 30 metacercariae. At 15 minute intervals the number of excysted metacercariae were counted and the results recorded. The experiment was terminated after 2½ hours. The remaining 30 control cysts were maintained in saline during the duration of the experiment and periodically examined for signs of excystation.

2.3 RESULTS

2.3.1 Viability of Eggs and Infection of Snails

The results of Experiment 1 are presented in Table 2.2 and Fig. 2.1.

Table 2.2 Percent hatch of eggs from progenetic metacercariae and from adults.

Source of eggs	No. eggs used	No. eggs hatched	Percentage hatch
Progenetic metacercariae (mysids)	25	24	96
Progenetic metacercariae (amphipods)	32	30	93
Adults (fish)	48	47	98

The percentage hatch of eggs from *C. parvum* was similar in all three groups. The first eggs began to hatch after 12-14 days and continued to hatch for a further five days. It was found that freshly laid eggs from progenetic *C. parvum* metacercariae develop and hatch at a similar rate to those from adult worms. The combined results of the percentage hatching of eggs from progenetic metacercariae in mysids and amphipods and from adults in fish are presented in Fig. 2.1.

Experiment 2: For comparative purposes, the results of Experiment 2 are also presented in Fig. 2.1.

Seventy-three percent of aged eggs from metacercarial cysts in mysids and 84% from amphipods had hatched over the three week period. The first eggs hatched within three to four days after commencement of the experiment while the remainder hatched over the following 16 days. Ninety-five percent of eggs collected from the faeces of fish used as controls in this experiment hatched within the normal 12-19 day period (cf. Experiment 1).

Experiment 3: The results of snail infection experiments using eggs from progenetic metacercariae and from adult flukes are presented in Table 2.3.

Fig. 2.1 Percent (cumulative) hatching of *Coitocaecum parvum* from amphipods, *Paracalliope fluviatilis* (open circles); aged eggs from progenetic metacercariae from mysids, *Tenagomysis chiltoni* (stars).

Dotted line from day 12 to day 19 represents hatching of eggs from Experiment 1. Combined results of freshly laid eggs (broken line).

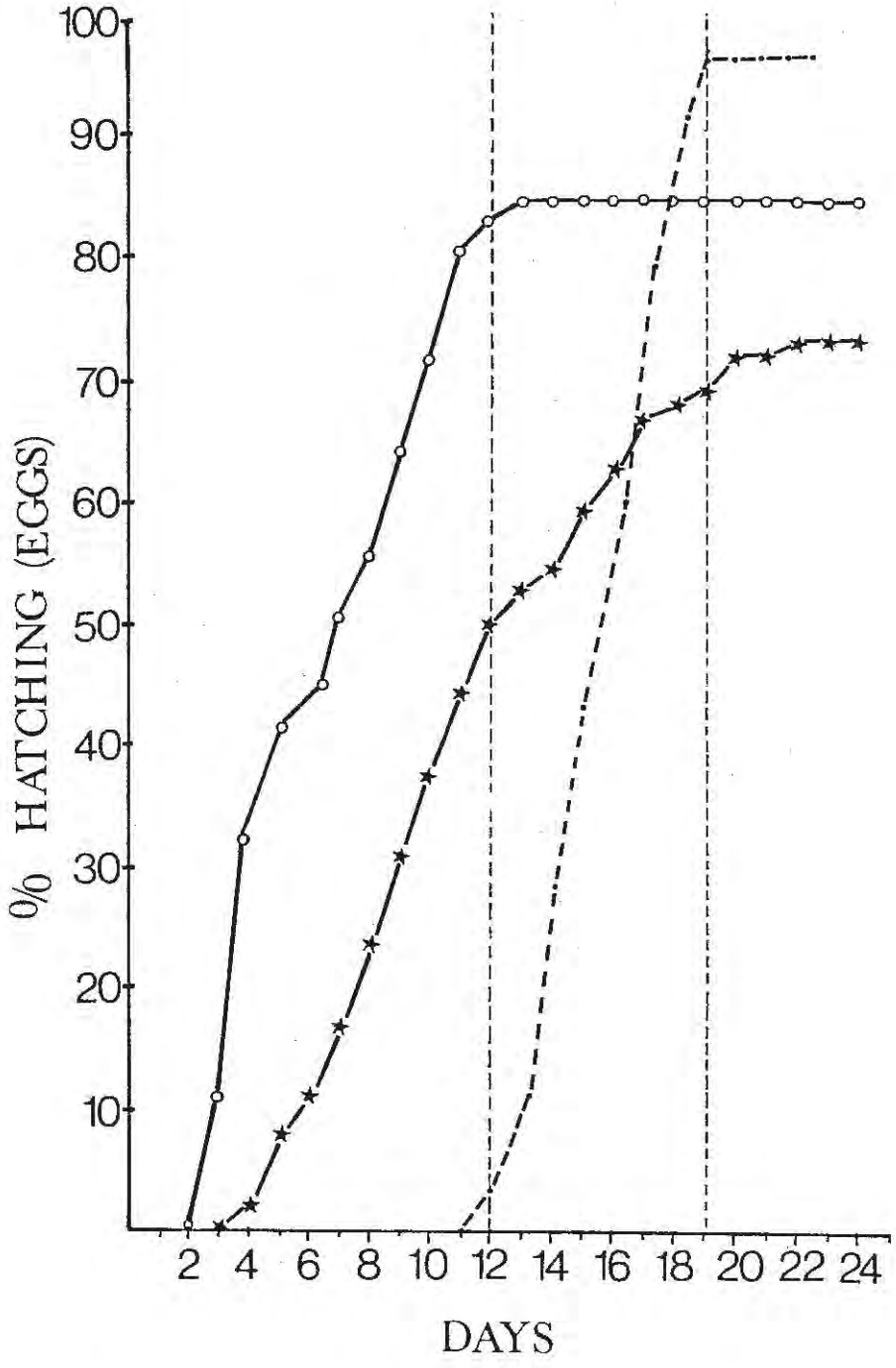

100
90
80
70
60
50
40
30
20
10
% HATCHING (EGGS)
2 4 6 8 10 12 14 16 18 20 22 24
DAYS

Table 2.3 Infection of snails.

Source of eggs	No. snails used	No. snails infected	Percentage infected
Progenetic metacercariae (mysids)	20	1	5
Progenetic metacercariae (amphipods)	20	2	10
Adults (fish)	20	2	10

Infected laboratory-reared snails were recovered from all three Petri dishes after isolation with eggs from progenetic metacercariae in mysids and amphipods and from adult flukes from fish hosts.

None of the 60 snails used in this experiment was found to be shedding cercariae at the termination of this experiment. However, on dissection, sporocysts were found in the tissues of five of the snails. Sporocysts were small (0.48 x 0.072 mm) and immature in comparison with those from natural and other experimental infections. As this experiment was only run for two weeks it is likely that sporocysts did not have enough time to complete their development.

2.3.2 Excystation of Metacercariae

Experiment 4: The results of excystation, Experiment 4, are presented in Table 2.4.

A total of 83 metacercariae was recovered from 20 autolysing mysids used in Experiment 4. Of these, 82 (99%) had excysted and were active when recovered from the tissues of the dead hosts. Only one metacercaria was still encysted at the termination of this experiment. A control group, consisting of 30 encysted metacercariae kept in saline, was still encysted after 24 hours.

Table 2.4 Excystation of metacercariae in autolysing mysids.

Petri dish	Number of mysids	Number of cysts	Number excysted	Percentage excysted
1	5	23	23	100
2	5	31	30	97
3	5	12	12	100
4	5	17	17	100
Control	-	30	0	0

Experiment 5: The results of Experiment 5 are presented in Table 2.5.

Table 2.5 Excystation of metacercariae after treatment with hepato-pancreas fluid from mysids.

Percentage excysted	Time in Minutes									
	15	30	45	60	75	90	105	120	135	150
Treated (N = 30)	40	60	65	65	70	70	75	79	81	85
Control (N = 30)	0	0	0	0	0	0	0	0	0	0

Eighty-five percent of the 30 metacercarial cysts treated with hepato-pancreas fluid from mysids excysted within 2½ hours after treatment. None of the 30 cysts kept in saline and used as a control excysted during this time.

Excystation was rapid during the first 30 minutes after treatment. Excysted metacercariae were observed moving on the bottom of the watch glass. Many of the remaining metacercariae excysted over the following 2¼ hours.

2.4 DISCUSSION

The majority of freshly laid eggs from progenetic metacercariae and from adult flukes develop and hatch within 12-19 days. The rate of development is similar to that seen for other species of trematodes (Kuris, 1980b; Vanoverschelde, 1980). Erasmus (1972) notes that most fluke eggs, which are undifferentiated when laid, take between 10 and 21 days to mature.

There were two differences in the rate and percentage hatch between freshly laid eggs from progenetic metacercariae and from aged eggs stored within the cysts. Firstly, almost all of the freshly laid eggs used in Experiment 1 developed and hatched (96, 93 and 98 percent) compared to only 73 and 84 percent of aged eggs. Secondly, freshly laid eggs began to hatch after 12 days whereas aged eggs from cysts started to hatch within 3-4 days. This may be explained in terms of the different degrees of development seen between recently emerged and aged eggs. Freshly laid eggs were undifferentiated and miracidia were visible in the eggs after 10-12 days. However, aged eggs which were collected from the cysts of progenetic metacercariae showed various degrees of development. Approximately 2% of these eggs contained active miracidia upon collection which probably hatched after only a few days in fresh water while the remaining eggs matured and hatched over the following 16 days.

It is likely that encysted progenetic metacercariae accumulate eggs over a considerable period of time (see page 80, Chapter III). Since there is no means of exit of eggs from the host, the length of time eggs remain within the cyst would depend on either predation or the host life span. It is therefore conceivable that some eggs remain within the cysts throughout almost the entire life span of the host (at least several months). Since it is found that freshly laid eggs developed in 12-19 days, it is assumed that some mechanism operating within the metacercarial cyst delays and prevents the premature hatching of eggs. Although this mechanism is not known, it is possible that delayed natural development or hatching may reduce the viability of ageing eggs. This may account for the slight decrease in the total percent hatch seen between freshly laid eggs and eggs which had been stored within metacercariae cysts. However, these findings are contrary to findings by other authors. De Giusti (1962) found that eggs from both progenetic *Allocreadium lobatum* in amphipods and from adults in fish took only 4-8 days to hatch at 72°F. There was no

difference in the hatching rate of eggs from these two sources.

Miracidia from progenetic *C. parvum* metacercariae are viable and capable of infecting the snail host *P. antipodarum*. However, the infectivity of miracidia from progenetic metacercariae and from adults is low when compared to other work (e.g., Moose, 1963; Goodchild and Fried, 1963; McClelland and Bourns, 1969; Hosier and Goodchild, 1970; Kuris, 1980b; Sluiters *et al.*, 1980). In these studies rates of infection achieved in laboratory-raised snails are usually between 38 and 100 percent. The reasons for low numbers of infections in snails in the present study are not known.

In this study *C. parvum* metacercariae were observed to undergo rapid excystation both on death of the mysid host, *T. chiltoni*, and following treatments with fluid from the hepato-pancreas of this host. Although there are many reports in the literature of experimental excystation of metacercariae following treatment by enzymes taken from warm-blooded definitive hosts, the situation seen in *C. parvum* appears to be unique. Excystation of metacercariae readily occurs on the autolysis and death of the crustacean host and, although the metacercaria itself will eventually die, by excysting in this way it allows the escape of its eggs from the confines of the cyst. This undoubtedly has many benefits for the further dispersal of eggs into the surrounding water. Once released into water eggs continue to develop and hatch. Miracidia emerging from these eggs are then capable of locating and infecting the first intermediate snail host, *P. antipodarum*.

In addition to the normal three host life history outlined in Chapter I, *C. parvum* has been found in this study to have the potential to abbreviate its life history through the production and dispersal of viable eggs by progenetic metacercariae. Such an abbreviated life history is likely to be of considerable benefit for the parasite species. Grabda-Kazubska (1976) notes three harzardous times during the life of a trematode. The first is in the location and penetration of snails by miracidia, the second is the finding of the second intermediate host by the cercariae, and the third is the transferral of the metacercariae to the final definitive host.

In a great many species, metacercariae do not become sexually mature until the definitive host is reached. In failing to be transferred to a

definitive host, such as in cases when the intermediate dies through natural causes or is eaten by a predator unsuitable for further development of the parasite, the metacercariae are completely lost from the parasite population. However, through progenesis of metacercariae, as is seen in *C. parvum*, a few individuals are able to continue the life history by the production and dispersal of viable eggs. Not only does the parasite benefit from the elimination of risk in transferring the metacercariae to the definitive host but also from an increase in the number of individuals in the population with the potential to reproduce (Grabda-Kazubska, 1976; Font, 1980). In addition to these advantages, progenetic metacercariae of *C. parvum* are presumed to reproduce by self-fertilisation (see page 43) and although the long term effects of continued selfing are not well understood (Clark, 1978), at least in the short term this has a number of benefits for the species. For example, progenetic metacercariae do not require the presence of other members of the species for continued reproduction. For further discussions on the benefits of self-fertilisation in parasites, see Clark (1978).

Despite the apparent advantages of progenesis in the life history of *C. parvum*, this species is still dependent, to a greater or lesser extent, upon the vertebrate definitive host. The life history pattern exhibited by *C. parvum* is similar to that of another trematode, *Alloglossidium progeneticum*. Font (1980) noted that in *A. progeneticum* progenetic metacercariae occur individually within cysts in the antennary glands of crayfish, while adults are found free in the intestine of catfish. Font (1980) gave two reasons for the continued retention of a vertebrate host in the life history of *A. progeneticum*. Firstly, he argued that eggs produced by metacercariae were prevented from exiting from the living crayfish due to the presence of a metacercarial cyst wall. Even though they may be released on death of the host, he considered that this was not an effective means for the dispersion of eggs. Secondly, progenetic metacercariae are only capable of self-fertilisation since the cyst wall acts as a barrier to copulation. Continued self-fertilisation may, in consequence, lead to homozygosity and perhaps in the long term at least reduces the capability of a parasite to survive changes in environmental conditions (also see Grabda-Kazubska, 1976). Retention of the vertebrate host, in this case, allows for continued cross-fertilisation and genetic exchange between individuals to occur.

In *Coitocaecum* only a small proportion of metacercariae become

progenetic while encysted in the second intermediate host. For this reason and the reasons outlined above by Font (1980), the abbreviation of the life history of *C. parvum* is considered only to be of secondary importance to the normal three host life history exhibited by this species.

2.5 SUMMARY

In this chapter, the possibility of an abbreviated life history in *C. parvum*, which may involve only two hosts, has been examined. Freshly laid eggs produced by progenetic metacercariae in mysids and in amphipods were found to hatch in distilled water after 12 days. Aged eggs which were recovered from the cavity of the metacercarial cyst were found to hatch after 3 - 4 days in distilled water. The percent of aged eggs that survived and hatched after prolonged storage in cysts was only slightly less than was seen for freshly laid eggs. Eggs of progenetic metacercariae from both mysids and amphipods were viable and capable of successfully infecting the first intermediate host, *P. antipodarum*.

Metacercariae were found to excyst both on death of the mysid host and following treatment with the fluid from the hepato-pancreas of the mysid host. Excystation of cysts under these conditions was considered to be a useful mechanism that aided in dispersal of eggs into the surrounding environment upon natural death of the intermediate host.

CHAPTER III

SOME FACTORS AFFECTING PROGENESIS IN *COITOCAECUM PARVUM*

3.1 INTRODUCTION

The occurrence of progenesis in the life histories of trematodes has been known since von Siebold (1835) first reported eggs emerging from metacercariae encysted in the ~~star~~fish *Astacus astacus* (cited by Stunkard, 1959). Since then there have been numerous reports of its appearance in various species of trematodes. Examination of the literature to date indicates that progenesis occurs in at least 18 families of Digenea worldwide.

Many authors have considered possible environmental and host-related factors which stimulate the manifestation of progenesis in metacercariae, however, as only a few experimental studies have been conducted, many of the theories on the causes of this phenomenon remain largely hypothetical (Grabda-Kazubska, 1976). The most common of these theories is that progenesis and egg production by trematodes result from the prolonged confinement of the metacercarial stage within the second intermediate host, combined with seasonal fluctuations in water temperatures (Wesenberg-Lund, 1934; Dollfus, 1959; De Guisti, 1962; Cable, 1965; Babero, 1972; Winstead and Couch, 1981). Other authors, such as McMullen (1938) and Srivastava and Ghosh (1969) considered progenesis to be a response to physiological changes in the host species resulting from the approaching 'senility' or hibernation of the host. MacFarlane (1951) and Baer and Joyeux (1961) believed the site of encystment within the second intermediate host to be an important factor relating to the nutritional requirements of the developing metacercariae. MacFarlane (1951) noted that progenetic *Stegodexamene anguillae* were most commonly found in sites "not subjected to pressure", such as the gonads. Finally, Buttner (1951), having bred ten successive generations of progenetic *Paralepoderma brumpti* through laboratory-reared snails, considered intrinsic heredity factors to be of greater importance than changing environmental conditions. The diversity of opinion as to the causes of this phenomenon seem to suggest

that different species of trematodes may be influenced by either or both environmental or intrinsic genetic factors (Font, 1980).

Preliminary studies conducted on the seasonal fluctuations of *Coitocaecum parvum* metacercariae in mysids suggested that the presence of progenetic metacercariae may have been influenced to some extent by seasonal variations in environmental conditions.

In this study it was considered that a continuation of the seasonal study may be useful in answering some of the questions raised by the previous work. For example: (1) Is progenesis in *C. parvum* metacercariae stimulated by changes in the environmental water temperature? (2) Is it stimulated by the presence of certain hormones released by hosts as they reach sexual maturity? (3) Are physiological changes that may occur in the hosts important as the hosts age and approach their death? (4) Is progenesis and the production of eggs by metacercariae the result of natural, prolonged confinement of the metacercariae within the second intermediate host? In an attempt to answer these questions, seasonal fluctuations in the prevalence and intensity of infestation, and the production of eggs by metacercariae were examined.

Fluctuations in the prevalence and intensity of metacercarial infections in crustacean hosts are known to be largely governed by seasonal invasions of cercariae and of the longevity of the host species (Cort, 1922; Rees, 1932; Blair, 1974; Chubb, 1979; Kitron, 1980; Kuris and Warren, 1980; Winstead and Couch, 1981). In order to interpret fluctuations in the prevalence and intensity of *C. parvum* infections in the mysid host it was considered necessary to have some understanding of both seasonal patterns of invasion by cercariae and population structure and reproductive activity of the snail and mysid host. The first of these was accomplished by determining the prevalence of *C. parvum* cercarial infections in the snail host, *Potamopyrgus antipodarum*. These results were used to indicate whether seasonal patterns may occur in the invasion of mysids by *C. parvum* cercariae. Secondly, information on the population structure and reproductive activity of snails and mysids was taken from previous work by Winterbourn (1970)b and Waite (1982).

3.2 METHODS

Regular monthly samples of snails and mysids were collected from

Timber Yard Point, Lake Ellesmere (Fig. 1.1A). Monthly water temperatures were also recorded using a thermometer placed at a depth of 300 - 350 mm.

3.2.1 Collection of Hosts

(a) Snails

Twelve samples of the snail host for *C. parvum*, *P. antipodarum*, were collected from Timber Yard Point between March 1981 and February 1982. Snails were collected in a hand net (a mesh size of 0.25 mm was used to ensure the smallest stages were collected) drawn across the substrate 0.5 metres from shore. On return to the laboratory the snails were killed within 1 - 5 days after collection. Each snail was placed in a watch glass in distilled water and examined under a dissecting microscope. The snails were gently crushed using a pair of fine forceps and the shell fragments removed to ensure that any larval trematodes present were not damaged. The number of snails infected with any larval trematode was recorded for each sample. Infections with *C. parvum*, identified on the basis of the morphology of the cercariae, were also recorded.

(b) Mysids

Twenty-two samples of the mysid *Tenagomysis chiltoni* were collected from Timber Yard Point from January to July 1980 and March 1981 to May 1982. Mysids were collected in a hand net (mesh size 0.25 mm) trawled 0.5 - 1.0 metres from shore. Mysids were examined for parasites within 1 - 3 days after collection. Each mysid was transferred to a watch glass and examined under a dissecting microscope at 10x magnification. The mysids were killed by decapitation using a pair of fine scissors. The length of mysids was always measured after death as live individuals were active and difficult to measure. The conventional distance used by Waite (1982) to determine total body length of mysids was from the tip of the rostrum to the tip of the telson. As mysids were decapitated in this study, measurements were taken from the dorso-posterior edge of the carapace to the tip of the telson. A sample group of 60 mysids of various sizes was measured using both of these methods and the measurements compared using Model II Regression Analysis. No significant difference was found between the two sets of measurements. Mysid lengths used in this study are given as a measurement of the distance between the dorso-posterior edge of the carapace to the tip of the telson. No attempt was made to convert these into lengths equivalent to those given by Waite (1982).

Following measurement, the degree of sexual development of each mysid was determined using the sexual characteristics outlined by Mauchline (1980) and Waite (1982). Individuals were classified as either juvenile, male adult, or female adult. The tissues of each mysid were then teased out into saline (0.75% NaCl in distilled water) and the number of *C. parvum* metacercariae recorded. The degree of sexual development of each metacercaria was determined and recorded as either immature or progenetic. Parasites defined as immature ranged from newly encysted to pre-egg bearing metacercariae, while progenetic metacercariae had eggs present within the uterus or cyst cavity.

The prevalence and intensity of all cysts (= immature + progenetic) and of progenetic metacercariae alone were recorded. In order to have some indication of age of the progenetic metacercariae the number of eggs in each cyst was also recorded.

The following statistical tests were performed: Student's t-test, Chi-squared analyses, and Wilcoxon's two sample test (Sokal and Rohlf, 1969; Parker, 1979; Steel and Torrie, 1980).

3.3 RESULTS

For ease of comparison, months were grouped into seasons as follows: autumn (March - May), winter (June - August), spring (September - November), and summer (December - February); these seasons are referred to in the following sections.

3.3.1 Water Temperature

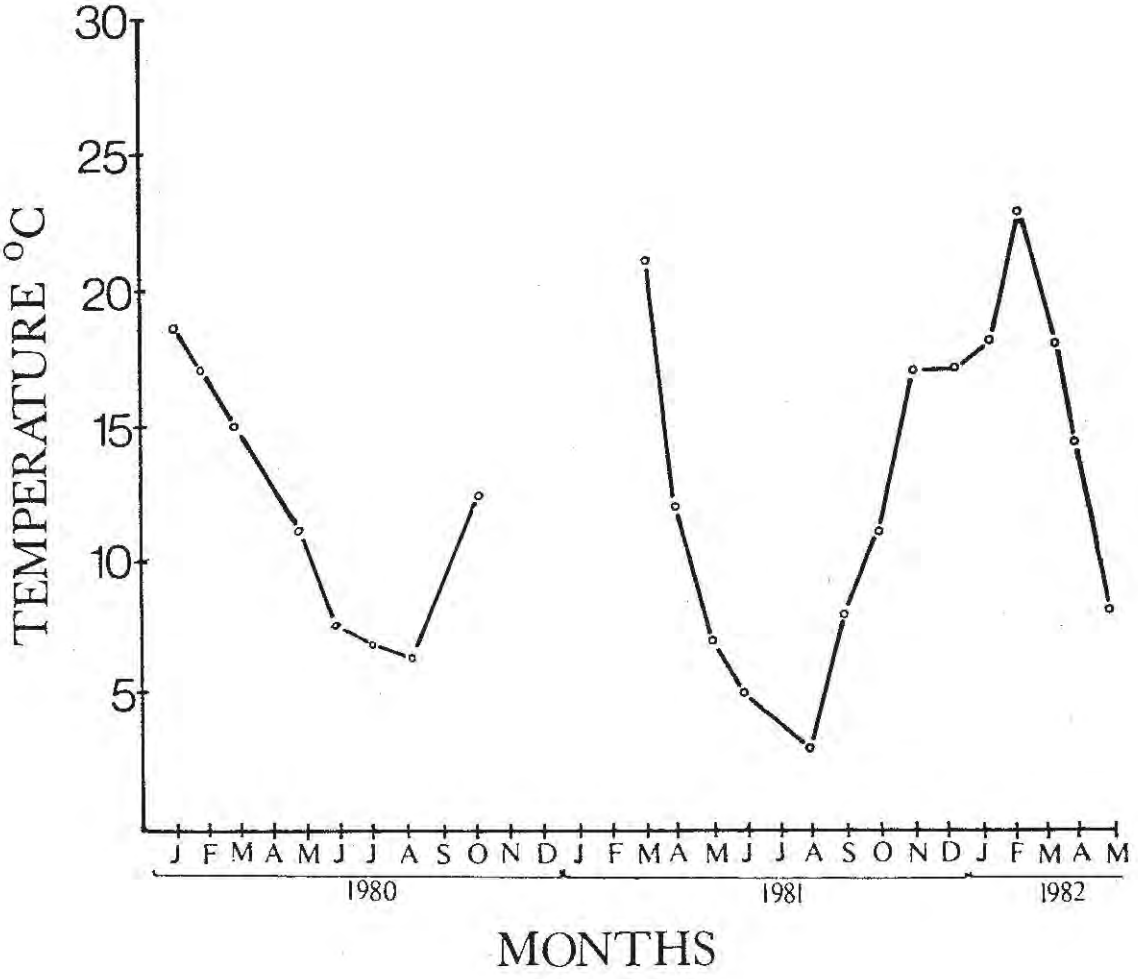
The water temperature recorded from Timber Yard Point, Lake Ellesmere at one or two monthly intervals is shown in Fig. 3.1. Water temperatures varied between 3.0°C recorded during winter (1981) and 22.0°C during summer 1981-82.

3.3.2 Seasonal Infection Patterns

(a) Snails

Two thousand seven hundred and seventy-two snails were collected

Fig. 3.1 Seasonal variation in water temperature at Timber Yard Point, Lake Ellesmere.


30
25
20
15
10
5
TEMPERATURE °C
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M
1980
1981
1982
MONTHS

from Timber Yard Point between March 1981 and February 1982. Eleven species of trematodes were found to infect *P. antipodarum* and included monostome, lophocercous, gymnocephalus and microcotylocercous cercariae and xiphidiocercariae. Cercariae were used in the identification of these types. Microcotylocercous cercariae were identified as *C. parvum* by experimental infection of mysid hosts and by comparison with cercariae derived from experimental infections of snail hosts (refer Chapter I, page 33). In addition to the cercariae, three unidentified species of metacercariae were also occasionally recovered from snails. Of the total number of snails examined, 251 (9%) were found to be infected with larval trematodes other than *C. parvum*, and 85 (3.1%) infected with *C. parvum*. Table 3.1 shows the number of snails examined and the percent infected during the 12 month sampling period. The prevalence of snails infected with all species of trematodes recovered and for *C. parvum* alone are presented separately.

A seasonal trend in the levels of infection in snails is apparent from Table 3.1. The prevalence of infection decreased from late autumn through winter. From early spring to summer the infection levels in snails gradually increased to reach the highest prevalence (28%) recorded during February (1982). A similar seasonal pattern was seen in the prevalence of *C. parvum*. Infection levels remained constant during autumn to early winter 1981. Levels were lowest in mid-winter and no infected snails were recorded during August. Gradually, from early spring to summer the proportion of snails infected with *C. parvum* increased to reach the highest levels (4.3%) recorded during February (1982).

(b) Mysids

Twenty-two samples comprising a total of 5012 mysids were collected from Timber Yard Point between January to July 1980 and March 1981 to May 1982. Of these, 2331 (46.5%) were found to be infected with metacercariae of *C. parvum*. Two hundred and three (8.7%) of the infected mysids were carrying progenetic metacercariae.

The number of mysids sampled, number infected, and total number of metacercariae collected each month over the sampling period are shown in Appendix I. In addition, the number of mysids infected with progenetic metacercariae and number of progenetic cysts collected are also presented in Appendix I.

Table 3.1 The number of *Potamopyrgus antipodarum* sampled, infected, and prevalence of infection (%) with all species of larval trematodes and with *Coitocaecum parvum* alone from March 1981 to February 1982. Timber Yard Point, Lake Ellesmere.

Month	No. snails sampled	No. infected All spp.	Prevalence (%) All species	*C. parvum*
1981				
March	328	43	13.1	3.0
April	319	29	9.1	3.4
May	381	40	10.5	3.1
June	321	29	9.0	3.4
July	239	13	5.4	1.2
August	223	7	3.1	0
September	259	22	8.5	1.9
October	128	10	7.8	1.5
November	284	40	16.9	1.4
December	183	23	12.5	2.1
1982				
January	207	41	19.8	3.4
February	139	39	28.0	4.3

(i) Prevalence of infection. The prevalence of mysids infected with *C. parvum* (all cysts) and with progenetic metacercariae was determined from Appendix I and the results presented in Figure 3.2.

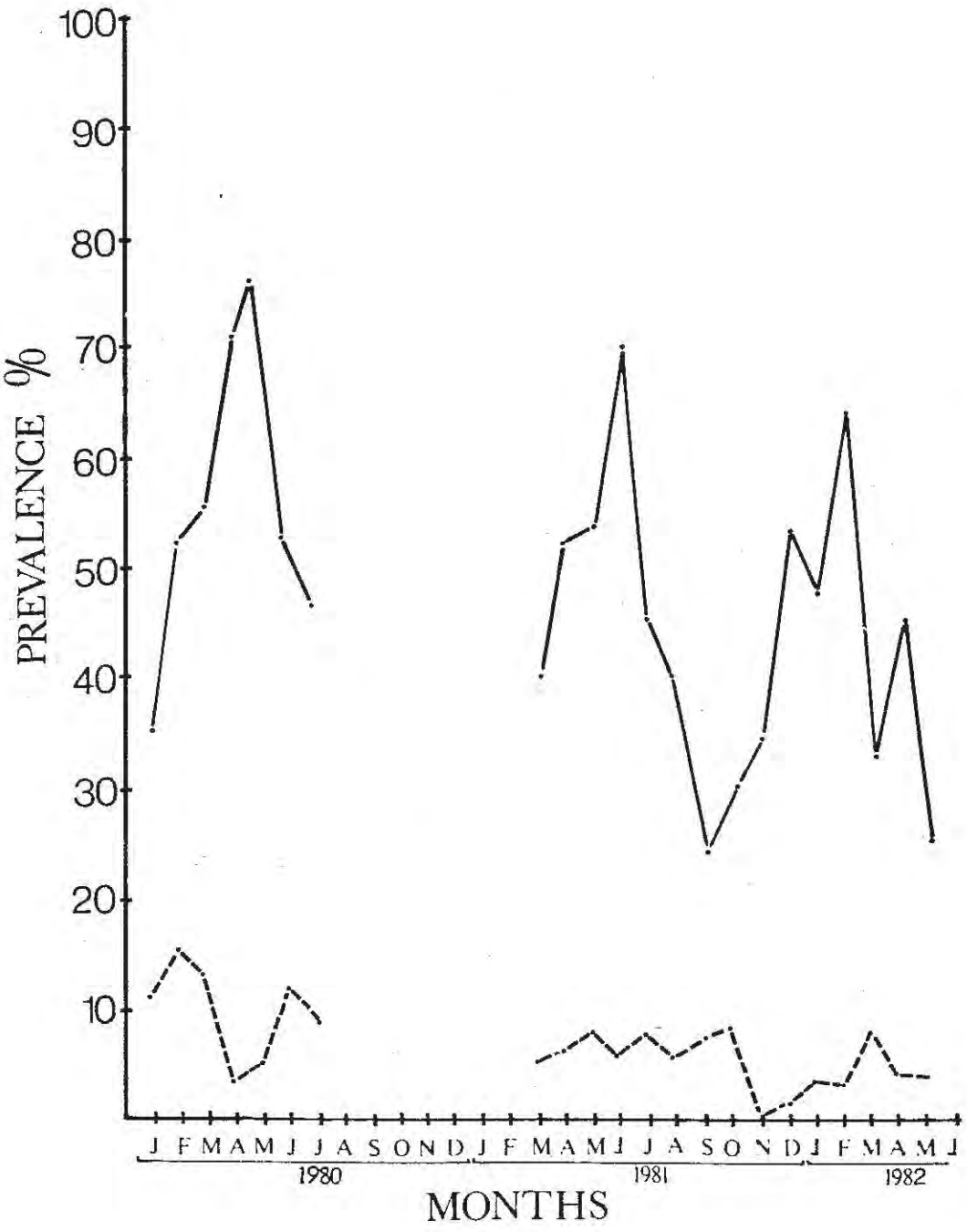
All cysts. Although the prevalence of infection fluctuated markedly with seasons, metacercarial cysts were present in mysids all year round. There were three peaks in the prevalence of infected mysids with the first occurring during autumn (1980). A gradual increase (by 40%) in the prevalence of infection from early summer reached its peak by mid-autumn (1980). This was followed by a decline in prevalence during early winter after which time sampling stopped. The second peak occurred, shortly after sampling commenced, over the autumn of 1981 when the prevalence of infection rose 30%. From autumn to winter the prevalence declined to reach its lowest during September (1981). However, the third increase (by 40%) occurred during the summer of 1981-82, resulting in a peak in prevalence during late summer 1981-82. In contrast to previous months, the prevalence of infection declined towards the end of summer and, although a smaller increase (14%) occurred during mid-autum (1982), it was not of the magnitude seen during similar months in previous years.

Progenetic cysts. Although metacercariae of *C. parvum* were present in mysids all year round, progenetic metacercariae were not (see November 1981, Fig. 3.2). Even so, there was no distinct seasonal pattern to the prevalence of infection. Over the January - July 1980 sampling period prevalence of mysids carrying progenetic metacercariae was highest during summer and winter and lowest during autumn. However, this pattern was not repeated during the 1981-82 period. From the end of summer 1981, through to the following spring, the prevalence of mysids infected with progenetic metacercariae rose gradually from 4.8 to 8.0 percent but, unlike the previous year, there was no decrease in prevalence during autumn 1981. The prevalence dropped from 8% in early spring to 0 one month later (October - November 1981). Over the rest of spring and summer the prevalence gradually rose to a peak of 8.5% during late summer, and was followed by a slight decrease during autumn 1982.

In summary, the prevalence of infection with progenetic metacercariae was seen to peak in mid-summer and again in late autumn of 1980, but peaks peaks also occurred in early spring 1981 and in early autumn 1982. No distinct, consistent seasonal trend was observed.

Fig. 3.2 Seasonal fluctuations in the prevalence of mysids infected with *Coitocaecum parvum*.

All cysts (solid line); progenetic metacercariae (broken line).


100
90
80
70
60
50
40
30
20
10
PREVALENCE %
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
1980
1981
1982
MONTHS

(ii) Intensity of infection. The mean intensity of all cysts and of progenetic cysts was determined for samples from March to May (1981-82) and the results presented in Fig. 3.3. Samples taken during January to July 1980 have not been included in this section as sample sizes of mysids were too small to yield any useful results ($n \leqslant 100$).

All cysts. The mean intensity of cysts per infected mysid varied considerably over the period of sampling. During autumn 1981 the intensity of infection increased from 2.2 to 3.5 cysts per mysid. Although this increase was not great there was a significant difference in the mean intensity of cysts per mysid between March and May samples as shown by a lack of overlap in 95% confidence limits. During winter (1981) the intensity declined to reach the lowest level by early spring. From the middle of spring to late summer there was a rapid increase in the intensity of infection. During summer and autumn (1982) the number of cysts per host varied markedly. Although the mean intensity of cysts per mysid collected during these months was between 4 and 7, a few hosts had accumulated as many as 60 metacercarial cysts. This variation in the intensity of infection is reflected in the magnitude of the 95% confidence limits attached to the means.

Progenetic cysts. The mean intensity of progenetic cysts fluctuated between one or two per host over the 15 month sampling period. There was no discernible seasonal pattern in mean intensity as shown in Fig. 3.3. No significant differences in intensity were found between any consecutive pairs of months from March to October 1981 (Wilcoxon's two sample test, $P > 0.05$). Sample sizes were too small to test for December to May 1981-82 ($n \leqslant 4$).

Proportion of cysts exhibiting progenesis. The proportion of all cysts that were progenetic was determined for each month from March (1981) to May (1982) and the results presented in Fig. 3.4. For statistical purposes the results from three months were grouped to correspond with the following seasons: autumn (1981), winter (1981), spring (1981), summer (1981-82), and autumn (1982). Chi-squared tests were performed between consecutive seasons.

Fig. 3.3 Seasonal fluctuations in the mean intensity of *Coitocaecum parvum* per host.

All cysts (solid line, with 95% confidence limits attached to the means); and progenetic metacercariae (dotted line).

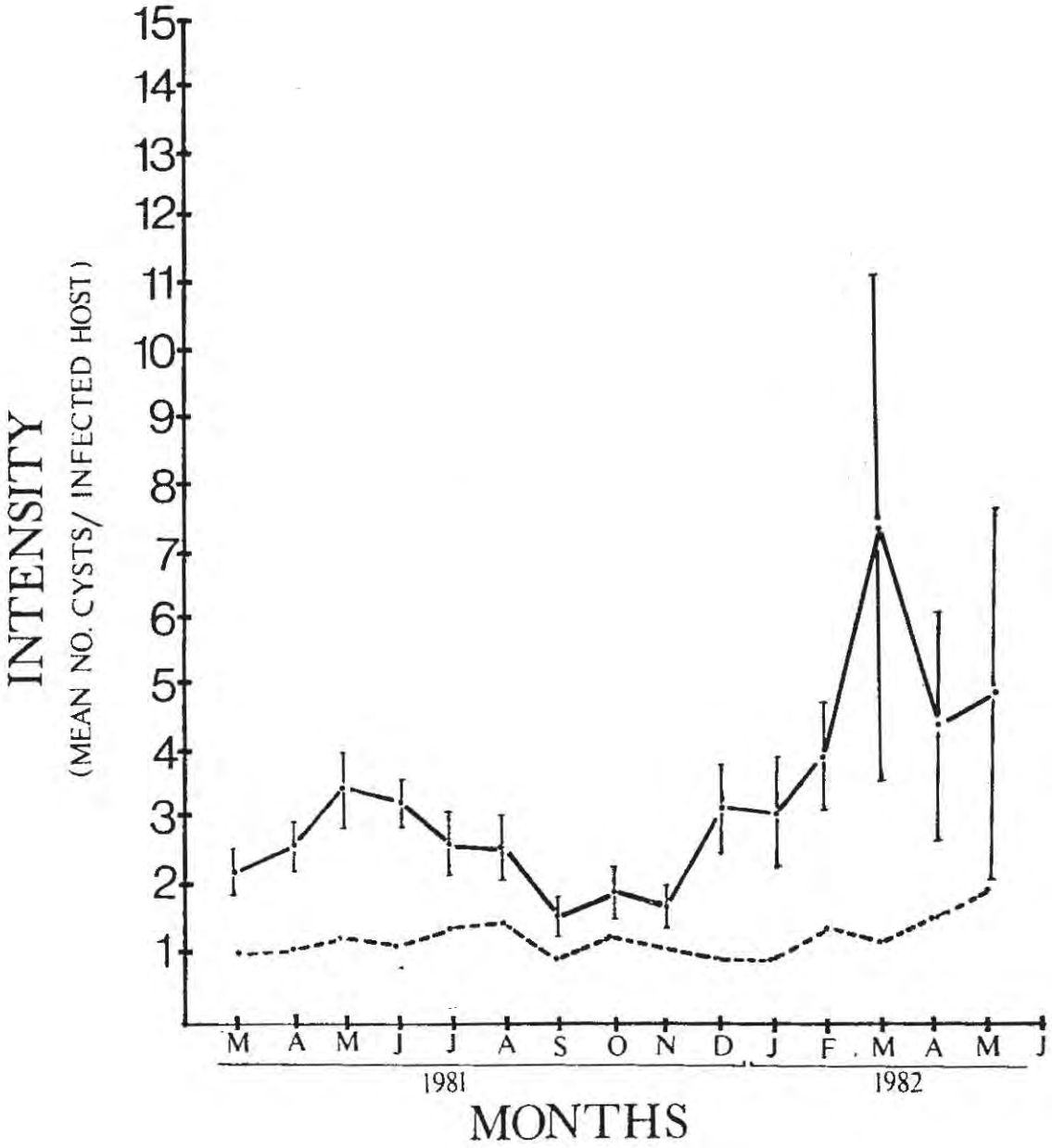
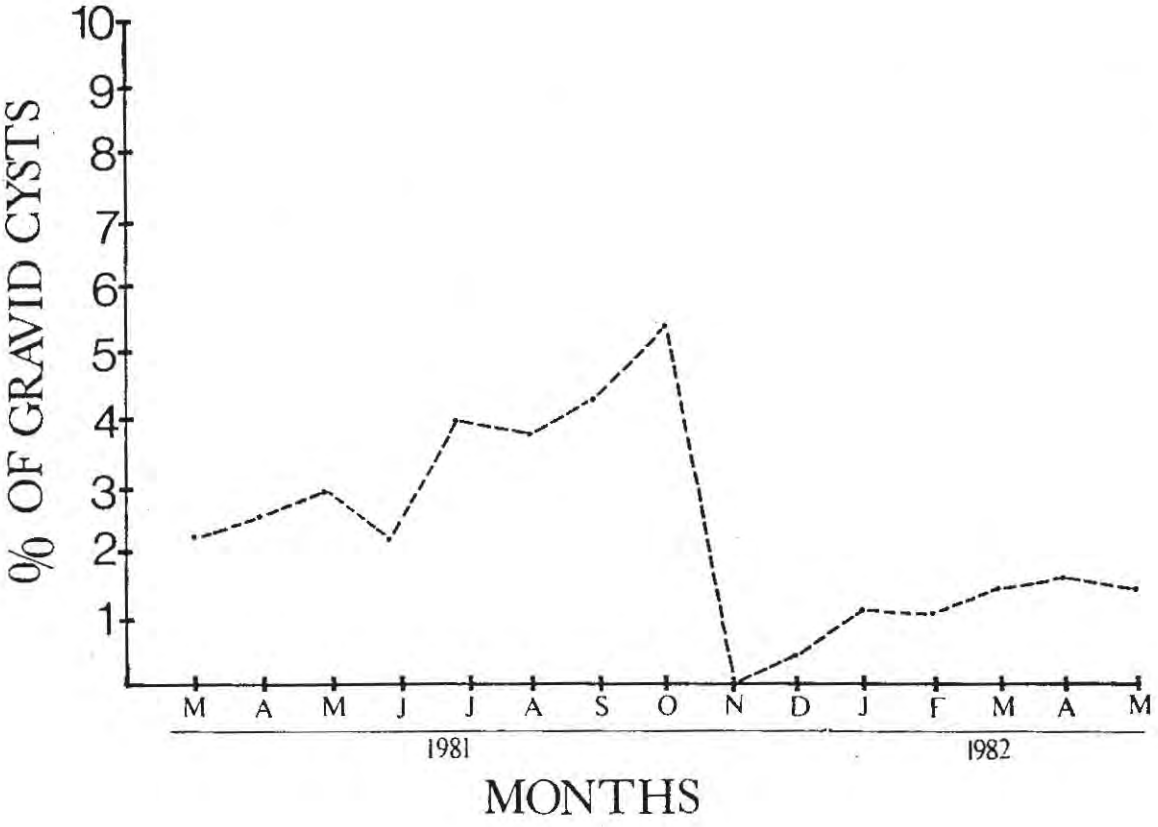

INTENSITY
(MEAN NO. CYSTS/ INFECTED HOST)
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
M A M J J A S O N D J F M A M J
1981
1982
MONTHS

Fig. 3.4 Seasonal fluctuations in the proportion of progenetic *Coitocaecum parvum* metacercariae in the parasite population.


% OF GRAVID CYSTS
10
9
8
7
6
5
4
3
2
1
M A M J J A S O N D J F M A M
1981
1982
MONTHS

There was no significant difference between percentage of progenetic cysts recovered in autumn and winter, 1981 ($\chi^2 = 0.00713$, $P > 0.05$), winter and spring, 1981 ($\chi^2 = 1.196$, $P > 0.05$), or summer 1981-82 and autumn 1982 ($\chi^2 = 0.629$, $P > 0.05$). However, there was a significant difference between the percent of all cysts that were progenetic collected during spring 1981 and summer 1981-82 ($\chi^2 = 7.763$, $P < 0.05$).

The number of progenetic metacercariae in the parasite population increased from autumn to the middle of spring 1981, followed by a marked drop from late spring to early summer. From summer to autumn the level again continued to increase.

(iii) Mean number of eggs per cyst. The mean number of eggs present in metacercarial cysts was determined for the sampling period from March 1981 to May 1982. The results are presented in Table 3.2.

The mean number of eggs present per cyst was highest during late autumn (May 1981), winter and spring (1981), and slightly lower in summer (1981-82).

The number of eggs recovered from progenetic cysts in mysids ranged between 1 and 98. Three cysts containing over 90 eggs were recovered during the entire sampling programme. All of these were collected during winter months.

3.3.3 Host Sex

During sampling, the sex of each mysid was recorded to determine if host sex was an important factor in the appearance of progenesis in metacercariae. All mysid samples were pooled and mysids classified into three groups - male, female, and juvenile. The prevalence of all cysts, and prevalence and intensity of progenetic cysts in hosts of each group were determined. Chi-squared analyses were used to test between the presence and absence of infection (all cysts) and between the prevalence of male, female, and juvenile hosts infected with progenetic cysts. Wilcoxon's two sample test for non-parametric distributions was used to determine if there were differences in the intensity of progenetic cysts in male, female, and juvenile hosts. Two values are given for prevalence

Table 3.2 Mean number of eggs per progenetic cyst for the 15 month sampling period.

Month (1981-82)	No. of progenetic cysts	Total no. of eggs	$\bar{x}$ S.D.
March	9	95	10.5 ± 10.8
April	20	230	11.5 ± 7.7
May	23	688	29.9 ± 26.1
June	20	528	26.4 ± 19.7
July	14	449	32.0 ± 24.6
August	10	246	24.6 ± 19.3
September	8	182	22.7 ± 11.7
October	14	329	23.5 ± 19.6
November	0	-	- -
December	1	23	23 -
January	3	58	19.3 ± 29.2
February	3	24	8.0 ± 5.3
March	5	80	16.0 ± 13.2
April	3	36	12.0 ± 11.5
May	2	7	3.5 -

of infection (Chi-squared) and intensity of infection (Wilcoxon's two sample test). The first represents comparisons between males and females and the second between adults and juveniles. 'Adult' was designated to represent pooling of male and female data.

(a) Prevalence of infection

(i) All cysts. The prevalence of infection in male, female, and juvenile groups is given in Table 3.3. No significant difference was found between the percent of male and of female infected with *C. parvum* (χ^2 = 0.158, P > 0.05). However, there was a highly significant difference between the prevalence of infection in adults and juveniles (χ^2 = 107.1, P < 0.001). A greater percentage of male and female mysids than juveniles was found to be infected with *C. parvum* metacercariae.

Table 3.3 Prevalence of *Coitocaecum parvum* in male, female, and juvenile mysids.

Sex	No. examined	No. infected	Prevalence (%)	χ^2 1 d.f.
Male (5.1 - 12.0 mm)	729	376	51.5	N.S.
Female (5.1 - 12.0 mm)	2088	1061	50.8	S ***
Juvenile (< 5.00 mm)	1519	525	34.6	

*** Highly significant difference (P < 0.001). N.S. Not significant.

(ii) Progenetic cysts. The prevalence of infected male, female, and juvenile mysids carrying progenetic cysts is presented in Table 3.4. Again there was no significant difference between the percent of males and of females infected (χ^2 = 1.42, P > 0.05). There was a highly significant difference between prevalence of adults and of juveniles infected with progenetic cysts (χ^2 = 23.3, P < 0.001). Male and female mysids were more likely to be carrying progenetic cysts than juveniles.

Table 3.4 Prevalence of progenetic metacercariae in male, female, and juvenile mysids.

Sex	No. infected (all cysts)	Infected with progenetic cysts	Prevalence (%)	χ^2 1 d.f.
Male	376	32	8.5	
				N.S.
Female	1061	69	6.5	
				S ***
Juvenile	525	8	1.5	

(b) Intensity

The intensity of progenetic cysts in male, female, and juvenile hosts is given in Table 3.5. There was no significant difference between mean number of cysts per mysid for males and females and for adults and juveniles (Wilcoxon's two sample test, $t_S = 0.836$, $P > 0.1$, and 0.332, $P > 0.5$, respectively).

Table 3.5 Intensity of progenetic cysts in male, female, and juvenile mysids.

Sex	No. infected	Total no. progenetic cysts	Intensity	t_S value
Male	32	36	1.12	
				N.S.
Female	69	89	1.3	
				N.S.
Juvenile	8	9	1.12	

3.3.4 Length of Host

During sampling the length of each mysid was recorded in millimetres to determine if the appearance of progenetic cysts was related to the size of the host. The results from all mysid samples collected between March (1981) and May (1982) were pooled. Six size classes were designated and

each mysid placed in the corresponding size class. Size classes were as follows: 1 (0.0 - 2.0 mm); 2 (2.1 - 4.0 mm); 3 (4.1 - 6.0 mm); 4 (6.1 - 8.0 mm); 5 (8.1 - 10.0 mm); 6 (10.1 - 12.0 mm).

(a) Prevalence and intensity of infection

(i) All cysts. Prevalence of infection in mysids increased with increasing length of hosts (Fig. 3.5). Significant differences ($P < 0.05$) in prevalence of infection were found between the following size classes: 1 and 2 ($\chi^2 = 4.86$), 2 and 3 ($\chi^2 = 49.9$), 3 and 4 ($\chi^2 = 41.9$), 4 and 5 ($\chi^2 = 20.3$). No significant difference was found between size classes 5 and 6 ($\chi^2 = 1.98$, $P > 0.05$). The intensity of infection in mysids of each class increased with length of host up to size class 5. However, between 5 and 6, the intensity of infection dropped from 3.7 to 2.9. It is not known if this difference was significant as sample sizes were large and time did not permit the use of statistical tests on this data.

(ii) Progenetic cysts. The prevalence of mysids infected with progenetic metacercariae also increased with increasing length of hosts (Fig. 3.6A). Highly significant differences in the prevalence of infection were found between the size classes 3 and 4 ($\chi^2 = 25.7$, $P < 0.001$), 3 and 5 ($\chi^2 = 42.6$, $P < 0.001$), 3 and 6 ($\chi^2 = 47.2$, $P < 0.001$), 4 and 5 ($\chi^2 = 6.94$, $P < 0.01$), 4 and 6 ($\chi^2 = 13.2$, $P < 0.001$). No significant differences were found between size classes 2 and 3 ($\chi^2 = 1.29$, $P > 0.1$) and 5 and 6 ($\chi^2 = 3.68$, $P > 0.05$).

The mean intensity of progenetic cysts in mysids of various size classes also increased with an increase in the length of the host. Significant differences in the intensity of progenetic cysts per host were found between size classes 3 and 6 ($U_s = 97$, $P < 0.05$), 4 and 6 ($t_s = 9.583$, $P < 0.001$), and 5 and 6 ($t_s = 2.169$, $P < 0.05$). No significant differences were found between 3 and 4 ($t_s = 0.544$, $P > 0.1$), 3 and 5 ($t_s = 0.675$, $P > 0.1$), and 4 and 5 ($t_s = 0.019$, $P > 0.1$) (Wilcoxon's two sample test, Sokal and Rohlf, 1969).

(iii) Proportion of all cysts exhibiting progenesis. The percent of *C. parvum* cysts that were progenetic was determined for each size class. Again there was an increase in percentage of progenetic cysts in the

Fig. 3.5 Prevalence (solid line) and intensity (dotted line) of *Coitocaecum parvum* infections (all cysts) in six size classes of mysids. 1 = 0 - 2.0 mm; 2 = 2.1 - 4.0 mm; 3 = 4.1 - 6.0 mm; 4 = 6.1 - 8.0 mm; 5 = 8.1 - 10.0 mm; 6 = 10.1 - 12.0 mm.

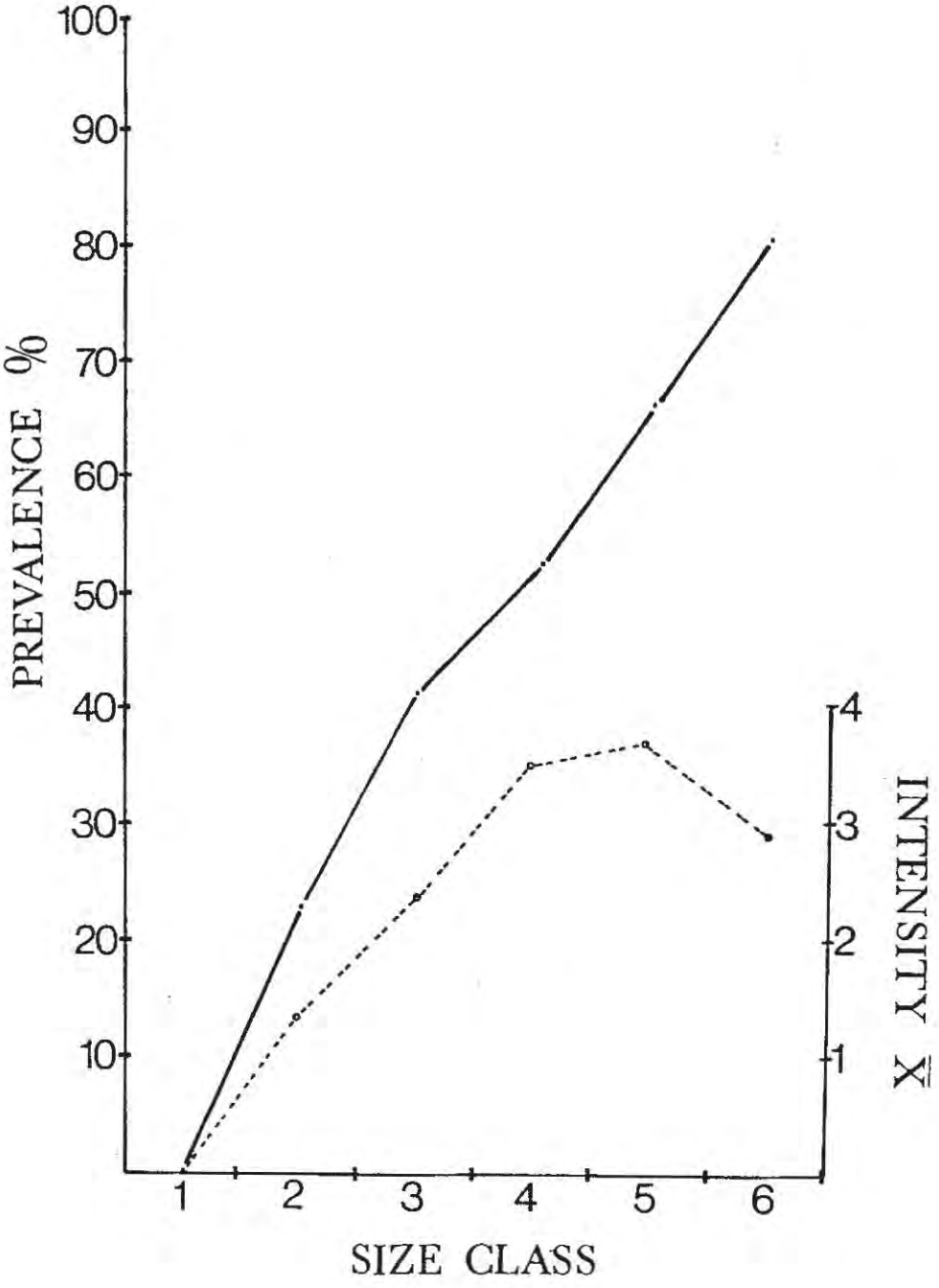

100
90
80
70
60
50
40
30
20
10
PREVALENCE %
4
3
2
1
INTENSITY X̄
1
2
3
4
5
6
SIZE CLASS

Fig. 3.6A Prevalence (solid line) and intensity (dotted line) of progenetic metacercariae in six size classes of mysids.

Fig. 3.6B Mean number of eggs per progenetic cyst in six size classes of mysids.

parasite population with an increase in the length of the host. In size class 3, 1% of metacercarial cysts exhibited progenesis compared to 2.3% in size class 4, 6.3% in size class 5, and 17% in size class 6. The Chi-squared test was used to compare the number of progenetic and immature cysts between combinations of all size classes. Highly significant differences ($P < 0.001$) were found between all combinations of size classes.

(b) Mean number of eggs per cyst

The mean number of eggs per progenetic cyst was also determined and plotted against length of host (Fig. 3.6b). There was an increase in the mean number of eggs per cyst from 15.5 to 40.9 with an increase in host length. Significant differences were found between size classes 3 and 6 ($t = 2.99$, $P < 0.05$), 4 and 6 ($t = 2.75$, $P < 0.05$), 5 and 6 ($t = 2.293$, $P < 0.05$), but not between 3 and 4 ($t = 0.736$, $P > 0.1$), 3 and 5 ($t = 1.51$, $P > 0.1$), or 4 and 5 ($t = 0.955$, $P > 0.1$).

3.4 DISCUSSION

3.4.1 Host Biology and Interpretation of Infection Patterns

In order to interpret seasonal changes in the infection of both the snail, *P. antipodarum*, and the mysid, *T. chiltoni*, with *C. parvum* it is necessary to have some understanding of the biology of these hosts.

(a) Snails

(i) Host biology. *Potamopyrgus antipodarum* is a small, freshwater gastropod commonly found in lakes, streams and rivers throughout New Zealand. Females are ovioviviparous and, although both sexes are normally present in approximately equal numbers, reproduction is primarily by parthenogenesis (Winterbourn, 1970b, 1980). Winterbourn (1970b) examined the population structure and reproductive activity of three populations of *P. antipodarum* from Palmerston North. He found that although snails reproduced throughout the year, the largest numbers of young snails were released during spring and summer. *Potamopyrgus antipodarum* was found to lack clearly defined reproductive periods, making it difficult to

distinguish between growth cohorts of snails. Although adult snails were present in ponds throughout much of the year, the numbers of adults in the population dropped to some extent during the cold winter months.

(ii) Seasonal patterns in infection. Seasonal fluctuations in the prevalence of *C. parvum* and other trematode larval stages in *P. antipodarum* from Timber Yard Point were similar to those seen in other trematode-molluscan studies (Chubb, 1979; Loker *et al.*, 1981). Peaks in prevalence were correlated with rising water temperatures (early spring, summer, and autumn) and lowest during cold winter months. In light of work conducted by Winterbourn (1970b) it is likely that the increases in prevalence seen during spring and summer were due to the recent recruitment of new infections in young snails. Likewise, decreases in prevalence during winter were probably due to a number of factors such as the mortality of ageing or infected snails, or to the failure of established infections to produce cercariae at this time. Analysis of seasonal changes in length and prevalence of trematode infections in *P. antipodarum* would have elucidated the causes of these fluctuations more clearly.

It is possible to surmise that invasion of mysids by cercariae from infected snails is most likely to occur during summer and autumn months. It is also possible that some new infections in mysids will also occur during parts of winter and early spring, although the magnitude is not likely to be as great as seen during warm summer and autumn months.

(b) Mysid hosts

(i) Host biology. The mysid *Tenagomysis chiltoni* occurs in fresh and brackish water commonly associated with coastal habitats around the Wellington and Canterbury areas (Chapman and Lewis, 1976). The first extensive work on the population structure and reproductive activity of *T. chiltoni* was conducted by Waite (1982) on mysids in Lake Ellesmere. He noted that successive, overlapping generations of mysids were present at various times of the year, and that two distinct periods of reproductive activity occurred during spring and summer. During much of the winter, the population consisted exclusively of a few, long-lived, sexually inactive mysids. As water temperatures increased during spring, these overwintering mysids became sexually active and produced large numbers of spring generation mysids. Within several weeks, subsequent to the release of the

spring generation, none of the overwintering mysids remained in the population. During the warm late spring and summer months, the spring generation matured rapidly and by the height of summer were sexually reproductive and releasing a summer generation of young mysids. Waite (1982) noted that towards the end of the summer months there was considerable overlap between these two generations making further distinction between them difficult; however, it appeared that within the following few months the spring generation disappeared from the population. A few summer generation mysids released late in summer did not become sexually mature but carried on through winter forming the basis of the long-lived, overwintering population.

(ii) Seasonal patterns in infection.

All cysts. Fluctuations in the prevalence of *C. parvum* infections in mysids are governed by two factors: (1) seasonal invasions by cercariae, and (2) the longevity and reproductive activity of the host species. In this study it was found that the prevalence of *C. parvum* infections in mysids was highest during autumn (1980), autumn (1981), and summer (1981-82) (Fig. 3.2). The intensity of infection in mysids was also highest during autumn (1981) and summer (1981-82) (Fig. 3.3). Increases in prevalence and intensity during these months was probably due to the invasion of spring generation mysids by cercariae escaping from infected snails. However, in late autumn (1981) the spring generation, which by this time had accumulated heavy infections with *C. parvum*, began to disappear from the population. This resulted in a decrease in prevalence and intensity of infection observed during these months (Fig. 3.2). The remaining summer generation mysids were still maturing at a time when the prevalence of cercarial infections in snails was beginning to decline (late autumn 1981) and probably only accumulated light metacercarial infections over the winter period. As they began to mature in early spring, they released a new spring generation of mysids which again accumulated heavy infections of *C. parvum* and resulted in an increase in the prevalence and intensity of infection observed in summer (1981-82).

Progenetic cysts. There was no consistent seasonal pattern in either the prevalence or intensity of progenetic metacercariae in mysid hosts from Timber Yard Point (Figs 3.2, 3.3). During January to July 1980 prevalence was highest in mid-summer and again in late autumn, while in

1981-82 the prevalence rose slowly through autumn, winter and early spring. Even though the prevalence of mysids infected with all cysts was decreasing during this time, the prevalence of progenetic cysts was not. This can be explained as follows. Spring generation mysids carrying heavy infections of *C. parvum* cysts, matured, bred and died within a few months. As a result, only a few metacercariae in these hosts reached sexual maturity (Fig. 3.2). The influx of newly encysted metacercariae during spring and summer may also have aided in lowering the proportion of progenetic to immature cysts as shown in Fig. 3.4. On the other hand, the summer generation mysids acquired only a few cysts but continued to survive as the overwintering population. Metacercariae within overwintering mysids matured slowly and even though water temperatures were lowest during this time, an increasing number of them reached sexual maturity before the massive die-off of their hosts in spring (Figs 3.2, 3.4).

The mean number of eggs per cyst was used in this study to give some indication of age of progenetic cysts. The largest numbers of eggs were present in metacercarial cysts recovered from overwintering mysids while the lowest numbers were found in cysts encysted in the spring generation mysids.

A discussion of the results presented in this section can be used in an attempt to answer the first question posed in the Introduction - Is progenesis in *C. parvum* metacercariae stimulated by changes in the environmental water temperature? There are possibly two effects that water temperature may have on metacercarial cysts; either (1) *warm* temperatures may increase the egg laying activity of metacercariae in summer, or (2) stimulate progenesis in metacercariae during *cold* winter months.

In this study three independent criteria were examined in an attempt to determine what effect changes in water temperature have on progenesis and production of eggs by *C. parvum* metacercariae. These were seasonal fluctuations in (1) the prevalence and intensity of progenetic infections in mysids, (2) the proportion of progenetic metacercariae in the parasite population, and (3) the mean number of eggs per progenetic cyst. In all three cases, values were highest during winter months and to a lesser degree during summer, even though two almost distinct populations of mysids were carrying the cysts during these times. It seems unlikely that during winter cold temperatures stimulate progenesis

in metacercariae in overwintering mysids, while in summer warm temperatures have a similar effect on cysts in spring generation mysids. It is therefore more probable that warm temperatures enhance the rapid maturation of cysts in summer while metacercarial cysts encysted in overwintering hosts remain largely unaffected by cold water temperatures.

In conclusion, progenesis in *C. parvum* metacercariae does not appear to be stimulated by changes in environmental water temperatures since metacercariae were found to produce eggs throughout much of the year. Warm temperatures may, however, aid in the rapid maturation of cysts during summer.

(iii) Host sex. The prevalence and intensity of progenetic metacercariae did not differ between male adult and female adult hosts. However, adult mysids (5.1 - 12.0 mm) were more likely to be infected with progenetic metacercariae than were juveniles (< 5.0 mm). In this chapter it was asked whether or not hormonal factors present within a particular sex of host may stimulate the appearance of progenesis in *C. parvum* metacercariae.

There are a number of reports in the literature on the effects of host sexual maturation on the maturation of their parasites (Symth, 1976). Miretski [(1951) cited by Smyth, 1976] demonstrated that the maturation of the helminth *Polystoma integerrimum* was apparently controlled by the production of gonadal hormones by its frog host. In crustaceans, oogenesis and particularly vitellogenesis are known to be under control of hormones of neurosecretory origin (Prosser, 1973). It may be possible that such hormones may also stimulate sexual maturity in some parasites. However, this does not seem to be the case for *C. parvum* since progenetic metacercariae were present, although not in equal numbers, in both sexually immature juveniles and mature adult mysids.

(iv) Host length. Prevalence and intensity of progenetic metacercariae, the proportion of progenetic metacercariae in the parasite population, and the mean number of eggs per cyst all increased with an increase in the size of the host. The third question posed in this chapter was, "Do physiological changes, which may occur in a host as it

ages and approaches death, stimulate the appearance of progenesis in *C. parvum* metacercariae?" McMullen (1938) examined seasonal patterns of progenetic development of three species of trematodes infecting the snail hosts *Stagnicola emarginata* and *Heliosoma companulatum smithii*. He found that during late summer the incidence of progenetic metacercariae was highest in snails that were approaching senility and death and concluded that progenesis was stimulated by physiological changes occurring in these hosts.

Although it was found in this study that large sized mysids were more likely to contain progenetic metacercariae than smaller individuals, it appears unlikely that this is related to changing physiological factors associated with ageing of hosts. If physiological factors were important in stimulating the appearance of progenetic metacercariae in mysids, then it may be expected that progenetic metacercariae would occur exclusively in large sized mysids. Clearly this is not so. Progenetic metacercariae were recovered on a few occasions from small, sexually immature mysids less than 4.0 mm, in addition to medium (4.0 - 6.0 mm) and large sized mysids (6.1 - 12.0 mm). It does not, therefore, appear that physiological factors account, in this study, for the appearance of progenesis in *C. parvum* metacercariae.

The final question asked in this chapter was whether or not the appearance of progenesis in *C. parvum* metacercariae was a natural result of the prolonged confinement of the cysts within the intermediate host. The evidence gathered during this sampling study seems to suggest that of all the possible causes examined, this seems to be the most likely explanation. Progenetic metacercariae were most commonly found in long-lived adult overwintering mysids, although they were also occasionally encountered in small juvenile hosts. *Coitocaecum parvum* cysts recovered from mysids during various times of the year were at various stages of development. This ranged from small, newly encysted metacercariae to medium-sized cysts with differentiated reproductive, digestive, and excretory systems, and finally to egg-bearing progenetic metacercariae (Fig. 1.6). It appears that growth is a gradual process which continues throughout the life of the metacercariae. The degree of sexual maturity attained by a metacercaria appears then to be dependent upon the duration of its encystment within the second intermediate host.

3.5 SUMMARY

In this chapter, some possible factors which may have stimulated the appearance of progenesis in *C. parvum* metacercariae have been examined.

Progenetic metacercariae were recovered from large male adult and female adult mysids but were also present, although not in equal numbers, in small juvenile hosts. Progenesis in *C. parvum* did not seem markedly influenced by water temperature, sexual maturity of hosts, or physiological changes that may occur as a host ages and nears its death. Rather, it was considered to be a result of natural growth of metacercariae due to prolonged confinement of the metacercariae within the tissues of the second intermediate host.

GENERAL CONCLUSIONS

1. The species of *Coitocaecum* commonly found in freshwater fishes in Canterbury was identified as *C. parvum* Crowcroft, 1944. Adults collected from naturally and experimentally infected fish resembled *C. parvum* in the extent of the vitellaria, position of the genital pore, structure of the male and female reproductive systems and in their size. They were distinct from the type specimens of *C. anaspidis* and *C. zealandicum*.

2. Life history stages were found to occur in a number of freshwater hosts in Canterbury such as bullies, Inanga, smelt, amphipods, mysids, and snails. The bully, *Gobiomorphus cotidianus*; smelt, *Retropinna retropinna*, and the mysid, *Tenagomysis chiltoni* are previously unrecorded hosts for *C. parvum*.

3. The possibility for an abbreviation of the normal three host life history of *C. parvum* has been examined. Progenetic metacercariae from both mysids and amphipods produced eggs which were viable and capable of infecting the first intermediate snail host, *P. antipodarum*. Eggs are probably released from crustacean hosts by the excystation of metacercariae after the death of the host.

4. Some possible factors that may have been responsible for the appearance of progenesis in *C. parvum* metacercariae were examined. Seasonal changes in water temperature did not markedly affect the production of eggs by metacercariae although during summer warm temperatures may aid in the rapid maturation of cysts. Sexual maturation of *C. parvum* did not appear to be stimulated by sexual maturation of the host or by physiological changes that may occur in the host as it ages. Progenesis in *C. parvum* was considered to be a result of natural prolonged confinement of cysts within the second intermediate host.

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APPENDIX I

Number of mysids examined, infected, total number of *Coitocaecum parvum* metacercariae collected from Timber Yard Point. The number of mysids infected with, and number of progenetic metacercariae collected, from January - July 1980, March 1981 - May 1982.

	Month	No. mysids examined	No. infected	Total no. parasites collected	No. mysids infected with progenetic cysts	Total no. progenetic cysts collected
1980	January	100	35	93	4	4
	February	100	52	130	8	19
	March	100	55	134	7	12
	April	76	54	120	2	3
	May	100	76	240	4	9
	June	100	52	173	6	15
	July	100	46	114	4	5
1981	March	460	185	405	9	9
	April	553	287	764	17	19
	May	438	233	813	18	23
	June	412	287	933	16	20
	July	289	129	353	10	14
	August	315	127	326	8	12
	September	334	80	141	6	6
	October	328	100	205	8	11
	November	339	115	212	0	0
	December	152	80	258	1	1
1982	January	178	85	263	3	3
	February	113	73	285	2	3
	March	148	47	345	4	5
	April	163	74	323	3	5
	May	114	29	143	1	2

APPENDIX II

A new crustacean and fish host for *Deretrema minutum* Manter, 1954 (Trematoda: Zoogonidae) from Canterbury.

INTRODUCTION

Crustaceans are common intermediate hosts for the larval stages of trematodes (Stunkard, 1967; Maren, 1979; Amin *et al.*, 1980). New Zealand has many crustaceans inhabiting its lakes and streams (Knox, 1969; Chapman and Lewis, 1976) which are likely to be potential hosts for parasitic larval stages. Unfortunately, the literature available on life histories of New Zealand trematodes is meagre and restricted only to work by MacFarlane (1936, 1939, 1945, 1951, 1952). It is well known that gastropods such as *Potamopyrgus antipodarum* act as a first intermediate host for numerous trematodes (Winterbourn, 1973; McArthur and Featherston, 1976). However, only one species of crustacean, the amphipod *Paracalliope fluviatilis*, has been recorded as a host for a larval trematode (MacFarlane, 1939).

During this study, the mysid *Tenagomysis chiltoni* was found to act as an intermediate host for two trematode species. These were identified as *Coitocaecum parvum* Crowcroft, 1944, and *Deretrema minutum* Manter, 1954. Some individuals of both of these species exhibited progenesis while encysted in this host. This paper is concerned with an examination of *D. minutum* from *T. chiltoni*, and a record of a new fish host for this trematode.

MATERIALS AND METHODS

Mysids were collected from Timber Yard Point, Lake Ellesmere. On return to the laboratory mysids were killed by decapitation and examined for cysts of *Deretrema minutum*. The site, prevalence, and intensity of infection were recorded. Cysts were teased from the tissues of mysids and placed in a watch glass in saline (0.75% NaCl in distilled water). Before fixation, metacercariae were excysted by gently rupturing the cyst wall with a blunt-ended needle. On release from the cysts,

metacercariae were seen to move slowly around the bottom of the watch glass indicating they had not been damaged by this method of excystation.

Metacercariae were either fixed in hot 10% formalin and stained with Delafield's Haemotoxylin or Gower's Carmine, or examined live in saline under coverslip pressure.

Measurements were taken from fixed ovigerous metacercariae. These measurements, along with dimensions of *Deretrema minutum* and *D. philippae* Hine, 1977 from the literature, are given in Table 1. The mean given in millimetres is followed by the range (in parentheses) and number of observations upon which measurements were based.

Fish caught in the net during sampling were brought back to the laboratory and examined for adult *Deretrema*. These fish were *Galaxias maculatus* and *Retropinna retropinna*.

RESULTS

Between October 1981 and February 1982, 1110 mysids were examined for cysts of *Deretrema*. Of these, 39 (3.5%) mysids were infected with this species. Usually only one cyst was found in each host although occasionally as many as three have been found. A total of 30 cysts was recovered, 14 (47%) metacercariae within these cysts had reached an advanced state of sexual maturity and were producing eggs. The number of eggs from a single worm varied between 15 and 193. Eggs remained within the uterus and were not passed out into the cyst lumen (Fig. 1) as is the case with progenetic metacercariae of *Coitocaecum*.

Cysts were transparent, oval, and measured 0.30 x 0.24 mm (0.10-0.48 x 0.18-0.40). The site of encystment of metacercariae was found to be specific, restricted only to the abdominal segments of the host. Cysts were never found in the thoracic region of mysids but always occurred within the musculature of the abdominal segments. Sixty-three percent of cysts were recovered from the third and fourth abdominal segments, while the remaining 37% were found in either the first, second or fifth segments.

Cysts of *D. minutum* were readily distinguishable from those of

Coitocaecum parvum, also found in *Tenagomysis chiltoni*. They were site specific and the excretory vesicle was always obvious as a black area within the cyst. Cysts of *C. parvum* were usually recovered from the haemocoel and thoracic appendages of the host but rarely from the abdominal segments of the abdomen.

Both freshwater fish, the galaxid *Galaxias maculatus*, and the common smelt *Retropinna retropinna*, caught from Timber Yard Point, Lake Ellesmere, were found to have adult *D. minutum* in the intestine. These were indistinguishable from progenetic metacercariae from mysids.

Description of Metacercariae (Fig. 1)

Ovigerous metacercariae ovoid, widest at acetabular region or slightly anterior. Body surface spined. Oral sucker sub-terminal, ventral. Prepharynx distinct, short, leading to well developed pharynx. Oesophagus short, divides anterior to acetabulum to form two saccular intestinal caeca which terminate at level of anterior edge of testes. Acetabulum, immediately pre-equatorial, smaller than oral sucker.

Testes large, paired, opposite and equal, posterior to acetabulum, regular, ovoid, longer than wide. Genital pore on left side of body anterior lateral margin opposite pharynx. Cirrus sac, elongate, arising dorsal to acetabulum, entirely enclosing seminal vesicle. Prostatic cells distinct in distal half of cirrus sac.

Ovary single, regular and ovoid, median between testes. Shell gland Y-shaped, posterior and to left of ovary. Uterus extensive, occupying hind portion of body, extending anteriorly to open at genital pore, contains many operculate eggs. Vitelline follicles in clusters of 5 - 7, lateral to acetabulum, posterior limit reaching anterior edge of testes. Excretory vesicle capacious, partially obscured by eggs.

DISCUSSION

The genus *Deretrema*, established by Linton (1910) for *D. fusillus*, belongs to the family Zoogonidae, sub-family Steganodermatinae. At present there are at least 16 species in the genus (Yamaguti, 1971, Hine, 1977). No life history stages other than the adult are known for any of

these species (Yamaguti, 1975). Another member of this sub-family, *Steganoderma messjatzevi* (Issaitschkow, 1928) Manter, 1947, was found by Uspenskaja (1952) [cited by Yamaguti (1970)] to have metacercariae which become progenetic in a crustacean host. Although worms had reached an advanced state of sexual maturity, eggs were absent. No other examples of progenesis in genera closely related to *Deretrema* are known.

Two species of *Deretrema* have been recorded from New Zealand. The first, *D. minutum* Manter, 1954, was described from the intestine of *Galaxias maculatus* (= *G. attenuatus*) Manter (1954). The second species, *D. philippae* Hine, 1977, was recovered from the gall bladder of *Galaxias divergens*.

Progenetic metacercariae from *Tenagomysis chiltoni* clearly belonged to *D. minutum* Manter, 1954. A discussion of differences between *D. minutum* and *D. philippae* was given by Hine (1977) and shall not be repeated here.

Both *G. maculatus* and *R. retropinna* from Lake Ellesmere have been found to be infected with *D. minutum*. The latter is a new definitive host for this trematode. *Galaxias divergens*, the definitive host for *D. philippae* is only found in inland and alpine streams (McDowall, 1978).

In summary, two new hosts are recorded for *Deretrema minutum* from Canterbury. The first, *Tenagomysis chiltoni* acts as an intermediate and harbours metacercariae which may become progenetic. The second, a definitive fish host, is the common smelt *Retropinna retropinna*. Adult *D. minutum* are found in the intestine of this host.

Table 1 Comparison between dimension of progenetic metacercariae from mysids and *Deretrema minutum* and *D. philippae*.

(mm)	Metacercariae (ovigerous) N = 12	*D. minutum* Manter, 1954 N = 12	*D. philippae* Hine, 1977
Body length	0.53 (0.32-0.65)	- (0.50-0.862)	1.99 (1.99-1.0)
width	0.22 (0.17-0.29)	- (0.185-0.308)	1.22 (1.28-0.66)
Oral sucker L	0.096 (0.075-0.114)	- (0.124)	0.031 (0.022-0.035)
W	0.086 (0.063-0.10)	- (0.0.87)	0.036 (0.022-0.041)
Prepharynx L	0.012 (0.009-0.014)	short -	absent -
Pharynx L	0.022 (0.014-0.028)	- (0.034-0.0.046)	0.012 (0.009-0.014)
W	0.033 (0.030-0.038)	- (0.036-0.054)	0.016 (0.009-0.017)
Oesophagus L	0.033 (0.020-0.045)	2.5x long as pharynx	0.029 (0.010-0.032)
Digestive caeca L	0.081 (0.072-0.096)	short -	- -
W	0.035 (0.030-0.043)	- -	- (0.097-0.32)
Acetabulum	0.079 (0.052-0.090)	- 0.076-0.12	0.029 (0.020-0.033)
	0.071 (0.036-0.090)	- -	0.031 (0.025-0.040)

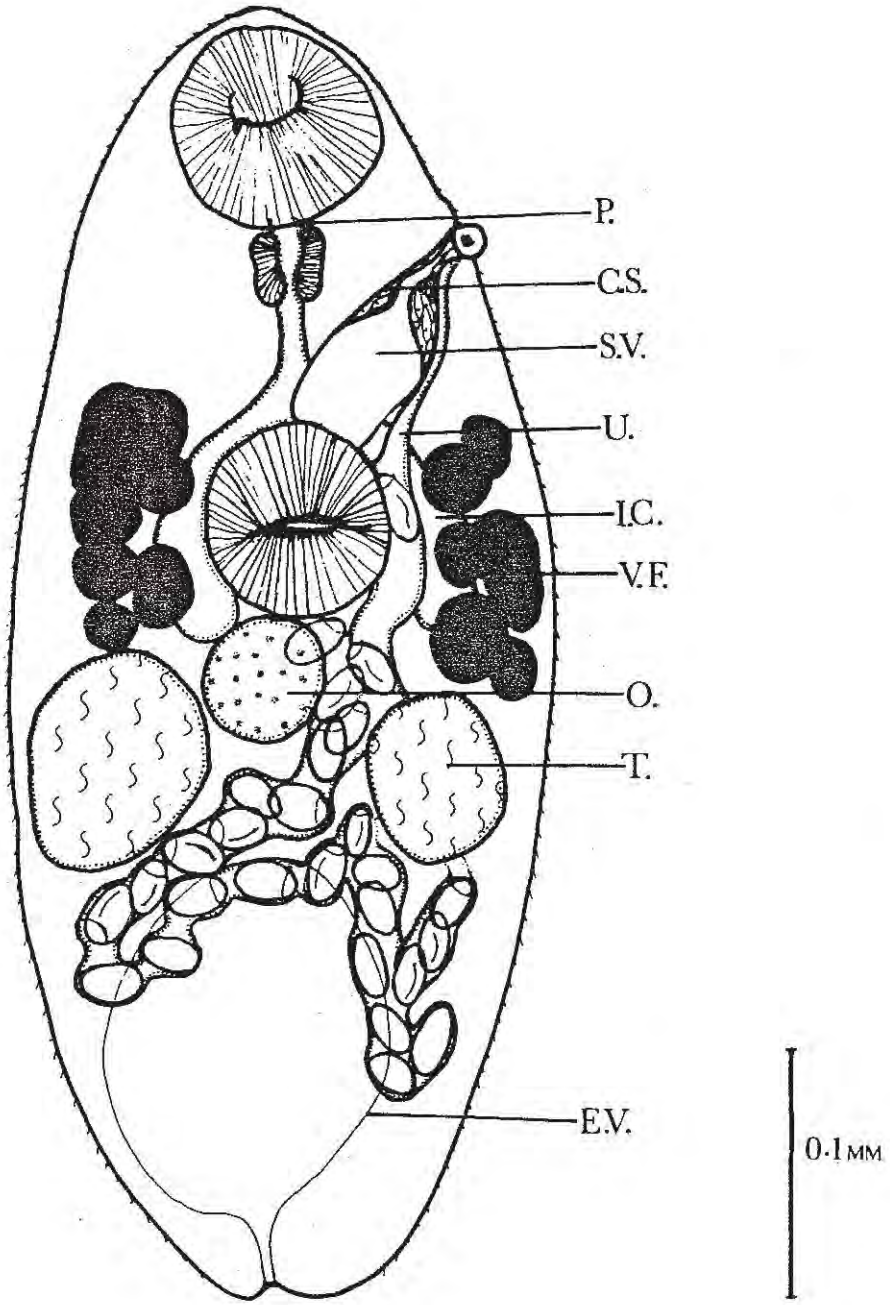
Cont'd....

Table 1 - Cont'd

(mm)	Metacercariae (ovigerous) N = 12	*D. minutum* Manter, 1954 N = 12	*D. philippae* Hine, 1977
Testes	0.095 (0.072-0.13)	- -	0.034-0.040 (0.013-0.047)
	0.066 (0.045-0.094)	- -	0.030-0.032 (0.007-0.041)
Genital pore	0.009-0.013 (0.012-0.016x0.08)	- -	0.007 (0.003-0.009)
Cirrus sac	0.12x0.03 (0.10-0.13x0.03)	0.17-0.025x0.042-0.051 -	0.06-0.008 (0.021-0.06x0.002-0.009)
Seminal vesicle	0.075x0.035 (0.055-0.10x0.016-0.04)	- -	0.063 (0.034-0.10)
Ovary	0.062 (0.055-0.078)	large -	0.025 (0.008-0.043)
	0.048 (0.034-0.055)	- -	- -
Shell gland	0.001x0.003	-	0.004
Eggs	0.030x0.015 (0.026-0.034x0.012-0.018)	- (0.034-0.041x0.019-0.022)	- (0.036-0.044x0.018-0.022)
Vitelline follicles	0.031-0.027 (0.022-0.036x0.020-0.047)	- -	0.013-0.014x0.009-0.013 (0.006-0.017x0.005-0.015)

Fig. 1 (Appendix II) *Deretrema minutum* (Manter, 1954), progenetic metacercaria, ex *Tenagomysis chiltoni*, Loc. Lake Ellesmere (N.Z.).

C.S. - cirrus sac; E.V. - excretory vesicle; I.C. - intestinal caecum; O - ovary; P - prepharynx; S.V. - seminal vesicle; T - testis; U - uterus; V.F. - vitelline follicle.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